Genetics of


spot
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hand
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science
muscle
ligament
arthritis
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immune
sprain
sclerosis
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rheumatoid
fracture
pharmacy
psoriasis
healthy
chronic
patient
dermatitis
diabetes
autoimmunity
antibody
profession
healthcare
disorders
arm
rheumatism
osteoarthritis
inflammation
disorder
ache
joint
acute
clinic
system
hurt
syndrome
sickness
people
injury
sbethoscope
physician
therapy
diagnosis
anatomy
treatment
autoimmune
dermatology
health
cramp
research
tendon
pain
medical
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allergy
arthrosis
lupus
care
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hospital
medication
trauma
disease


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In autoimmune diseases affecting millions, researchers pinpoint genetic loci harboring risk alleles

Autoimmune disorders associated with monogenic traits

Major features of monogenic autoimmune diseases.

Disease	Affected gene and function	Number of mutations	Main clinical features	Affected pathway	Mouse model
APS1	*AIRE* transactivator	>50	Hypoparathyroidism, Addison's disease, Candida, diabetes, ovarian failure	Self-antigen presentation and negative selection in thymus	KO
ALPS1a	*TNFRSF6* (FAS) membrane receptor	>40	Splenomegaly, lymphadenopathy, hypergammaglobulinemia, autoimmune diseases	Fas-mediated apoptosis	lpr/lpr-mouse KO
ALPS1b	*TNFSF6* (FASL) membrane-bound ligand	1	Systemic lupus erythematosus, lymphadenopathy	Fas-mediated apoptosis	gld/gld-mouse
ALPS2	*Caspase 10* cysteine protease	2	Adenopathy, hepatosplenomegaly, haemolytic anaemia	Lymphocyte apoptosis cascade	None presently available
IPEX	*Foxp3* transcription factor	10	Polyendocrinopathy, haemolytic anaemia, chronic diarrhoea, eczema	$CD4^+CD25^+$ regulatory T cell development	Scurfy-mouse
IL-2Rα deficiency	*IL-2Rα* cytokine receptor	1	Lymphadenopathy, chronic diarrhoea, hepato-splenomegaly, chronic lung disease, recurrent infections	$CD4^+CD25^+$ regulatory T cell development	KO

KO, knockout.

APS1= autoimmune polyendocrine syndrome o **APECED= autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy**

ALPS= autoimmune lymphoproliferative syndrome

IPEX=Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance

Genes implicated in both rare Mendelian and common immune disorders

Gene	Basic function	Mendelian disease with causative mutation	Common disease with susceptibility association
AIRE	Transcriptional regulator for tolerance to self antigens	APECED	Rheumatoid arthritis
FOXP3	Transcription factor for regulatory T cells	IPEX	Vitiligo
FAS (and related)	Pro-apoptotic	ALPS	Systemic lupus erythrematosis
PI3K (several genes)	Cell growth, proliferation, and survival	PASLI/APDS	PI3K inhibition ameliorates RA, SLE
CTLA4 (and related)	T cell inhibitory molecule	CHAI	Myasthenia gravis, alopecia areata

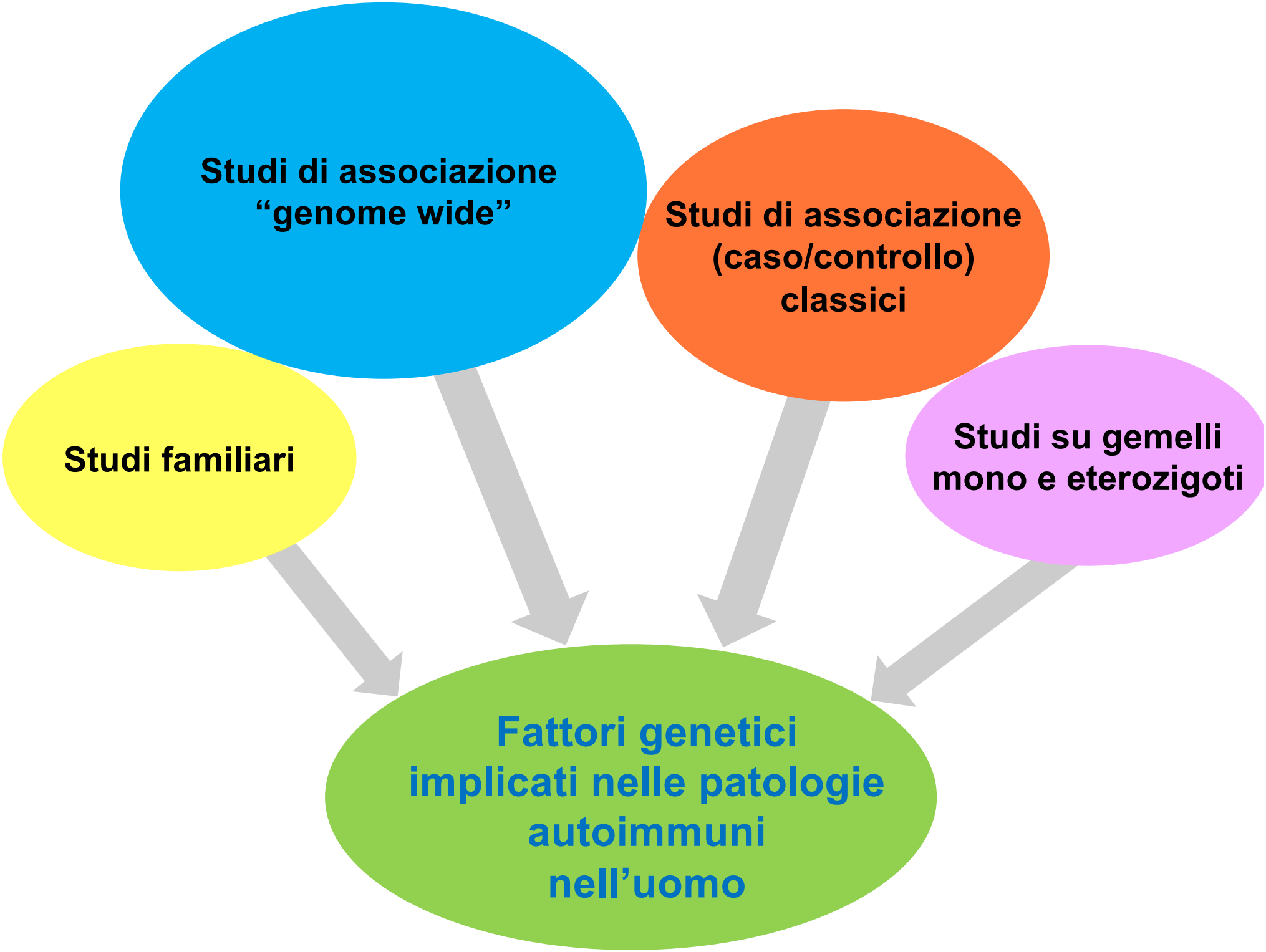
APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy;

IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome;

ALPS, autoimmune lymphoproliferative syndrome;

PASLI, p110δ-activation with senescent T cells, lymphadenopathy, and immunodeficiency;
APDS, activated p110δ syndrome;

CHAI, *CTLA4* haploinsufficiency with autoimmune infiltration.


Studi di associazione
“genome wide”
Studi di associazione
(caso/controllo)
classici
Studi familiari
Studi su gemelli
mono e eterozigoti
Fattori genetici
implicati nelle patologie
autoimmuni
nell’uomo

Approcci genetici per identificare i geni di suscettibilità per le patologie autoimmuni in modelli animali e nell'uomo

Genetica classica (forward genetics): manipolazione genetica di uno specifico gene (espressione per transgenesi; knockout e mutagenesi) e si osserva poi il fenotipo indotto

Genetica inversa (reverse genetics): si parte da un ceppo animale che spontaneamente sviluppa la patologia (o da coorti di individui malati rispetto ai sani) e si procede all'analisi di associazione per identificare i loci genici responsabili di quel fenotipo

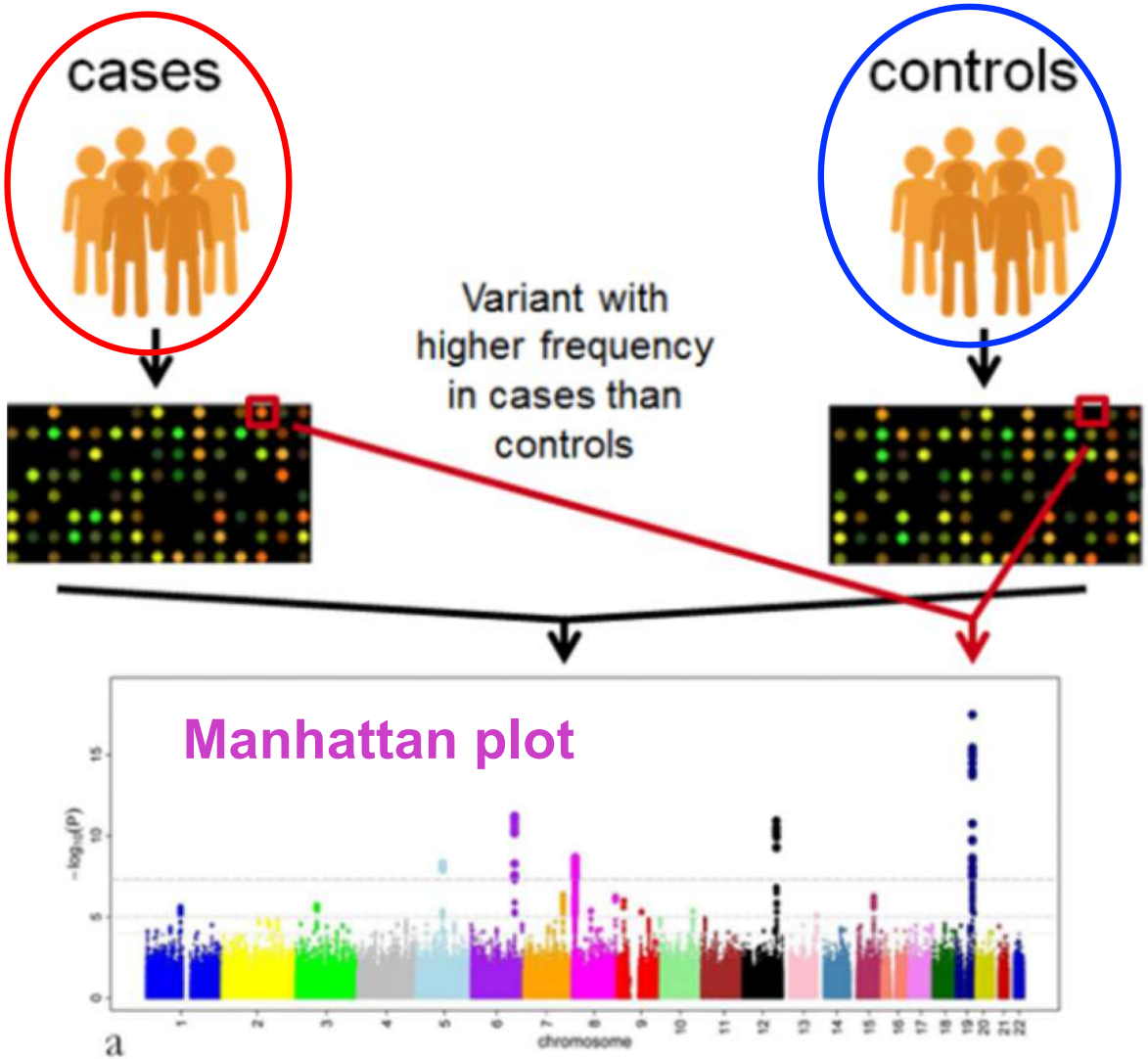
Approcci:

1. Candidate gene association studies (pre-GWAS studies)

2. Family-based linkage analysis

3. **GWAS=**genome wide association studies; Studi di associazione caso-controllo su tutto il genoma

GWAS are hypothesis-free methods for identifing associations between genetic regions (loci) and traits (including diseases)

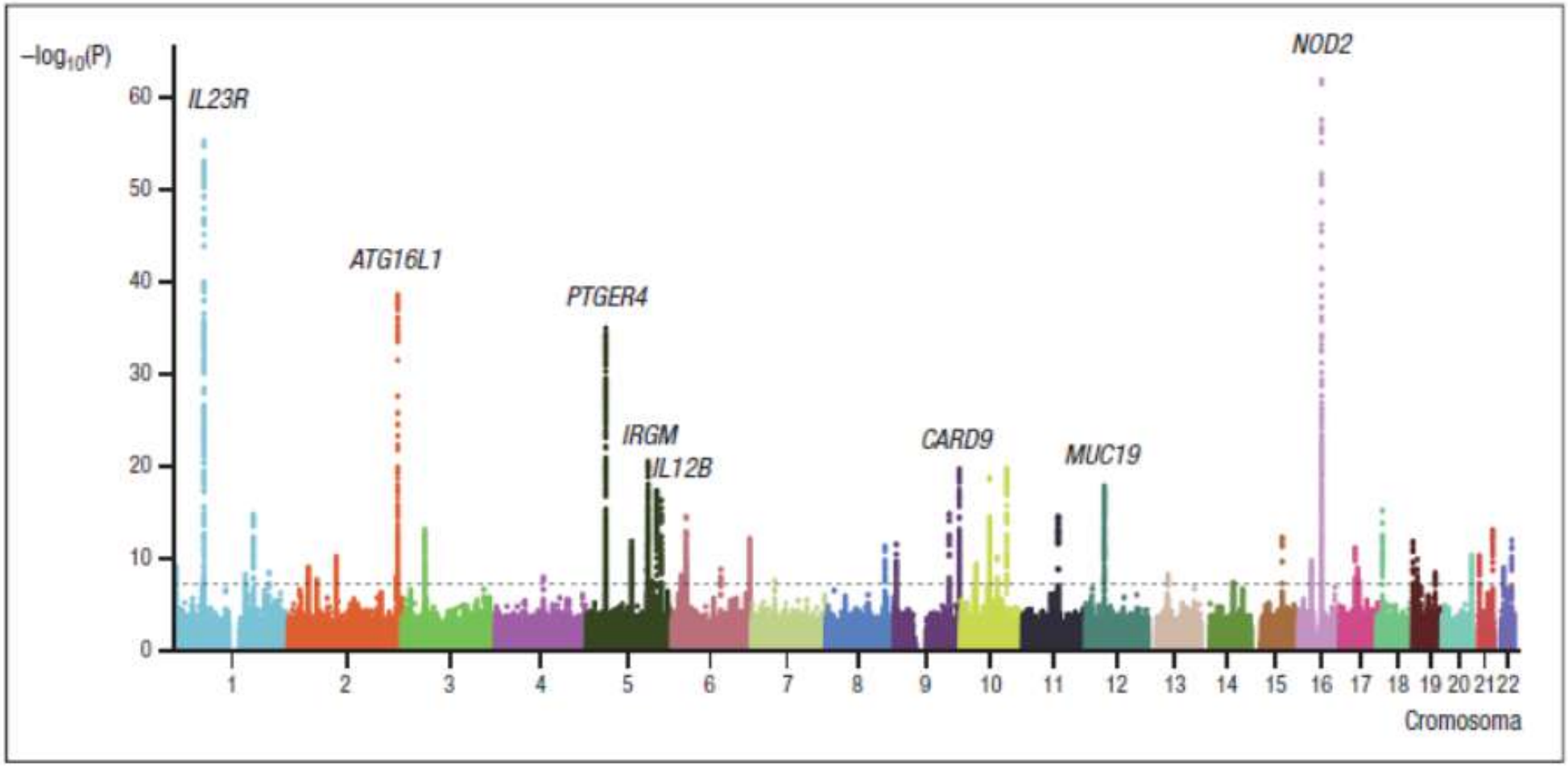


It has long been known that genetic variation between individuals can cause differences in phenotypes. These causal variants, and those which are tightly linked to their region of the chromosome, are therefore present at higher frequency in **cases** (individuals with the trait) than in **controls** (individuals without the trait). A typical GWAS study collects data to find out the common variants in a number of individuals, both with and without a common trait (e.g. a disease), across the genome, using **genome wide SNP arrays**. Variants associated with the disease, or within the same haplotype as a variant associated with a disease, will be found at a higher frequency in cases than in controls. **Statistical analysis** is carried out to indicate how likely a variant is to be associated with a trait.

As GWAS analyse common variants, usually typed on commercial SNP arrays, they do not generally identify causal variants. GWAS identify common variants which tag a region of linkage disequilibrium (LD) containing causal variant(s). Additional or follow-on studies are usually required to narrow the region of association and identify the causal variant. A p-value indicates the significance of the difference in frequency of the allele tested between cases and controls i.e. the probability that the allele is likely to be associated with the trait. GWAS results are often displayed in a Manhattan plot with -log10 (p-value) plotted against the position in the genome.

Manhattan plot

GWAS studies



Scatter plot che riporta gli alleli associati al rischio di **malattia di Crohn** che sono stati identificati mediante GWAS. Il grafico mostra i loci genici identificati mediante analisi di SNPs (single nucleotide polymorphisms) nei pazienti con Crohn rispetto ai controlli sani. L'altezza dei picchi riflette la significatività statistica delle associazioni. La linea orizzontale a puntini indica la soglia delle associazioni considerate significative (5×10^{-8}).

GWAS usati per costruire il network tra malattie autoimmuni ed i geni associati

Table 2

GWAS Studies used for network analysis

Phenotype	Cases	Controls	Analyzed SNPs	#SNPs reported (criteria)	Reference
CeD	778	1422	310 605	50 ($P < 10^{-2}$)	[41]
CD	382 (trios)	–	164 279	62 ($P < 10^{-3}$)	[49]
CD	393	399	92 387	139 ($P < 10^{-2}$)	[62]
CD	547	548	308 332	6 (top)	[63]
CD	547	928	302 451	1 (top)	[51]
CD	946	977	304 413	23 ($P < 10^{-4}$)	[50]
CD	94	752	72 738	4 ($P < 10^{-3}$)	[64]
CD	2000	3000	469 557	502 ($P < 10^{-3}$)	[35]
MS	931	2431	334 923	114 ($P < 10^{-3}$)	[44]
MS	978	883	551 642	44 ($P < 10^{-3}$)	[65]
Ps	318	288	313 830	3 ($P < 10^{-4}$)	[66]
RA	1522	1850	297 086	193 ($P < 10^{-2}$)	[34]
RA	625	558	203 269	14 ($P < 10^{-2}$)	[67]
RA (CCP+)	397	1211	79 853	205 ($P < 10^{-3}$)	[53]
RA	2000	3000	469 557	380 ($P < 10^{-3}$)	[35]
SLE	94	538	52 608	1 (top)	[68]
SLE	51	54	262 264	5 (top)	[69]
SLE	720	2337	265 648	35 ($P < 10^{-2}$)	[36]
T1D	1028	1143	534 071	88 (top)	[58]
T1D	2000	3000	469 557	102 ($P < 10^{-2}$)	[35]
T2D	1464	1467	386 371	102 ($P < 10^{-2}$)	[70]
T2D	105	102	115 352	72 ($P < 10^{-2}$)	[71]
T2D	640	674	80 044	89 (top)	[72]
T2D	124	295	82 485	125 (top)	[73]
T2D	500	497	315 917	7 (top)	[74]
T2D	1161	1174	315 635	97 ($P < 10^{-2}$)	[75]
T2D	661	614	392 935	50 ($P < 10^{-2}$)	[76]
T2D	1399	5275	313 179	48 (top)	[77]
T2D	3757	5346	393 453	65 ($P < 10^{-3}$)	[78]
T2D	307 (trios)	–	66 543	45 ($P < 10^{-3}$)	[79]
T2D	91	1083	70 987	5 (top)	[80]

Current Opinion in Immunology 2009, 21:596-605

Bassa penetranza dei geni associati

The ten autoimmune diseases with the highest number of GWAS studies registered at the GWAS catalog

Trait	EFO IDs	GWAS catalog					
		# Studies	# Cases	# Controls	# SNPs	# Loci	# Gene
Systemic lupus erythematosus	EFO_0002690	37	13,377	194,993	788	132[1]	439
Rheumatoid arthritis	EFO_0000685	37	22,628	288,664	421	>150[2]	249
Multiple sclerosis	MONDO_0005301	27	14,802	26,703	603	233[3]	479
Crohn's disease	EFO_0000384	27	12,924	21,442	411	>200[4]	265
Ulcerative colitis	EFO_0000729	25	12,366	33,609	295	>200[4]	184
Inflammatory bowel disease	EFO_0003767	12	25,042	34,915	387	>200[4]	238
Vitiligo	EFO_0004208	10	2,853	37,405	91	49[5]	80
Sjogren syndrome	EFO_0000699	10	1,599	658,316	48	25[6]	42
Grave's disease	EFO_0004237	8	4,487	629,598	74	12[7]	27
Behcet's syndrome	EFO_0003780	8	3,197	5,785	40	21[8]	35

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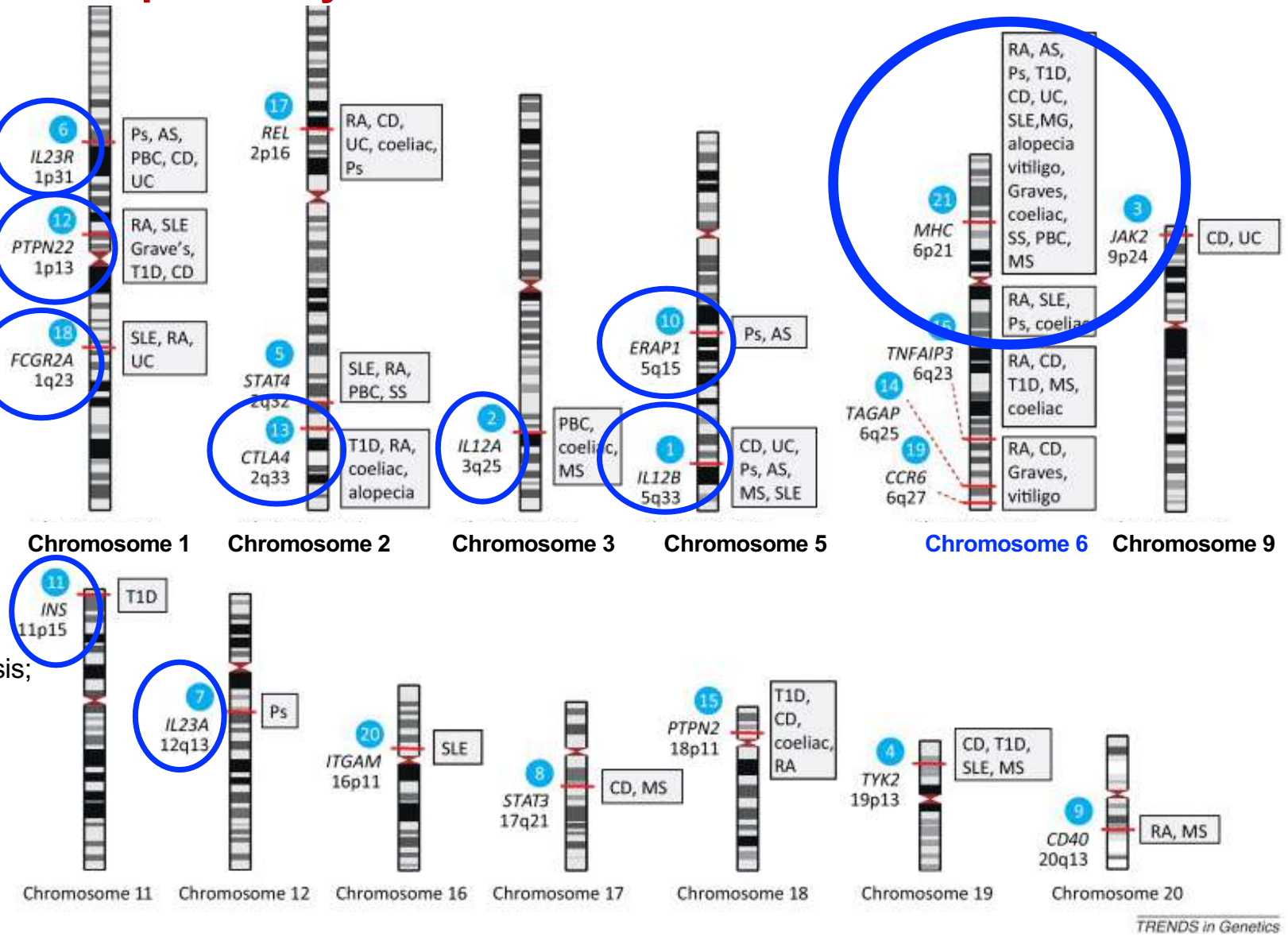
Displayed is the summary of information obtained from GWAS catalog,.With respect to GWAS catalog, this is the number of unique studies, the highest number of cases with corresponding number of controls, the number of unique variants reported the number of independent, associated genomic loci reported in the literature, the number of unique genes or gene combinations reported in the respective publications.

Examples of loci reported by GWAS in common autoimmune diseases



Alopecia areata;
AS, ankylosing spondylitis;
CD, Crohn's disease;
coeliac disease;
Grave's disease;
MG, myasthenia gravis;
MS, multiple sclerosis;
PBC, primary biliary cirrhosis;
Ps, psoriasis;
RA, rheumatoid arthritis;
SLE, systemic lupus erythematosus;
SS, systemic sclerosis;
T1D, type 1 diabetes;
UC, ulcerative colitis.

This is not an exhaustive list and illustrates the overlap seen at many associated loci for different autoimmune diseases and how genes encoding different components of pathways may be associated with a given disease using data from [6] and the National Human Genome Research Institute (NHGRI) Catalogue of Published Genome-Wide Association Studies (www.genome.gov/gwastudies/) where full details can be found. It should also be noted that, in the majority of cases, the genes listed are candidates based on the genomic locus that was associated, and causality has not been established except in a small number of instances. Knight JC 2013 Trends in genetics

Loci genici di suscettibilità per le malattie autoimmuni

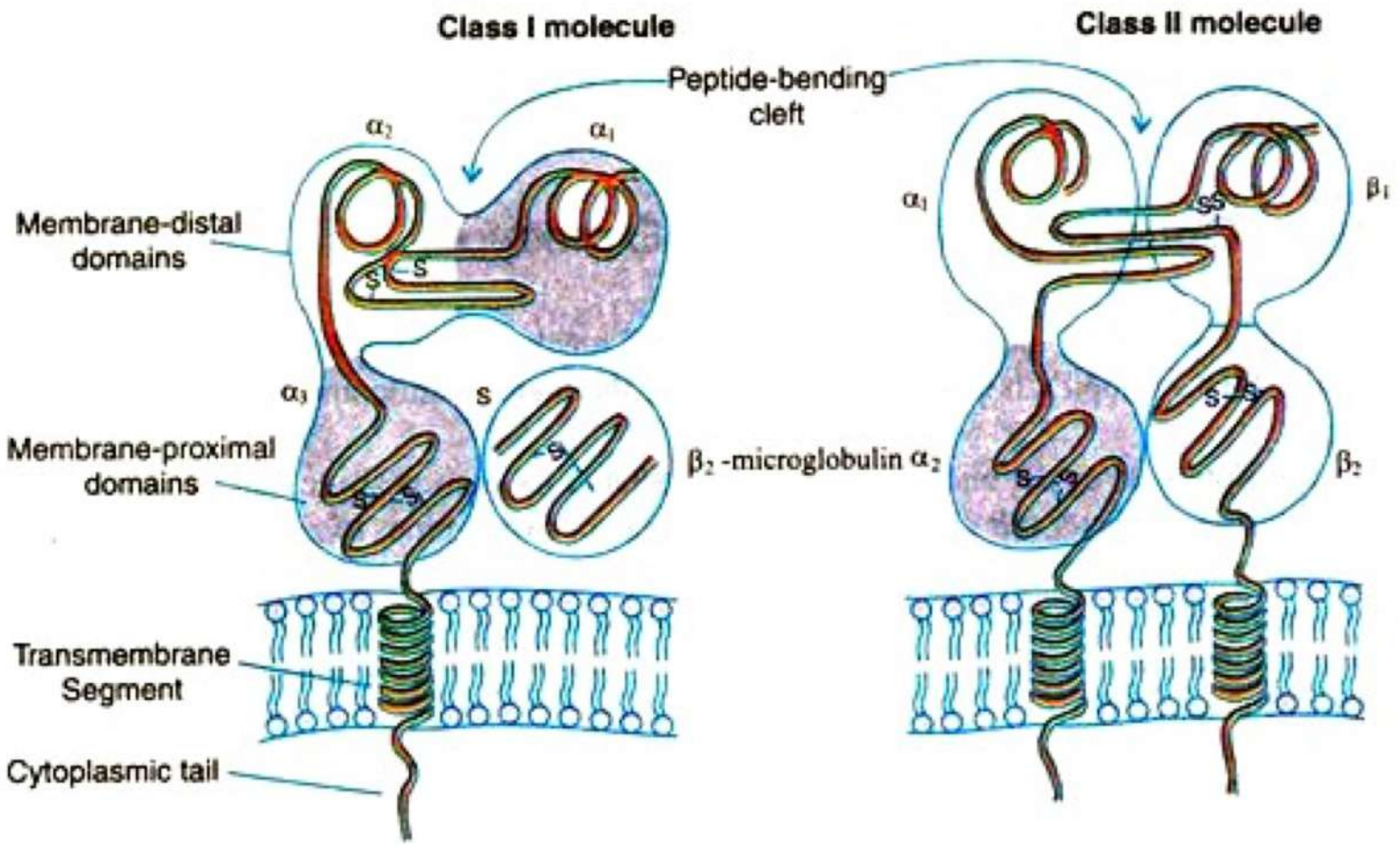
❖geni della regione HLA

❖geni non-HLA

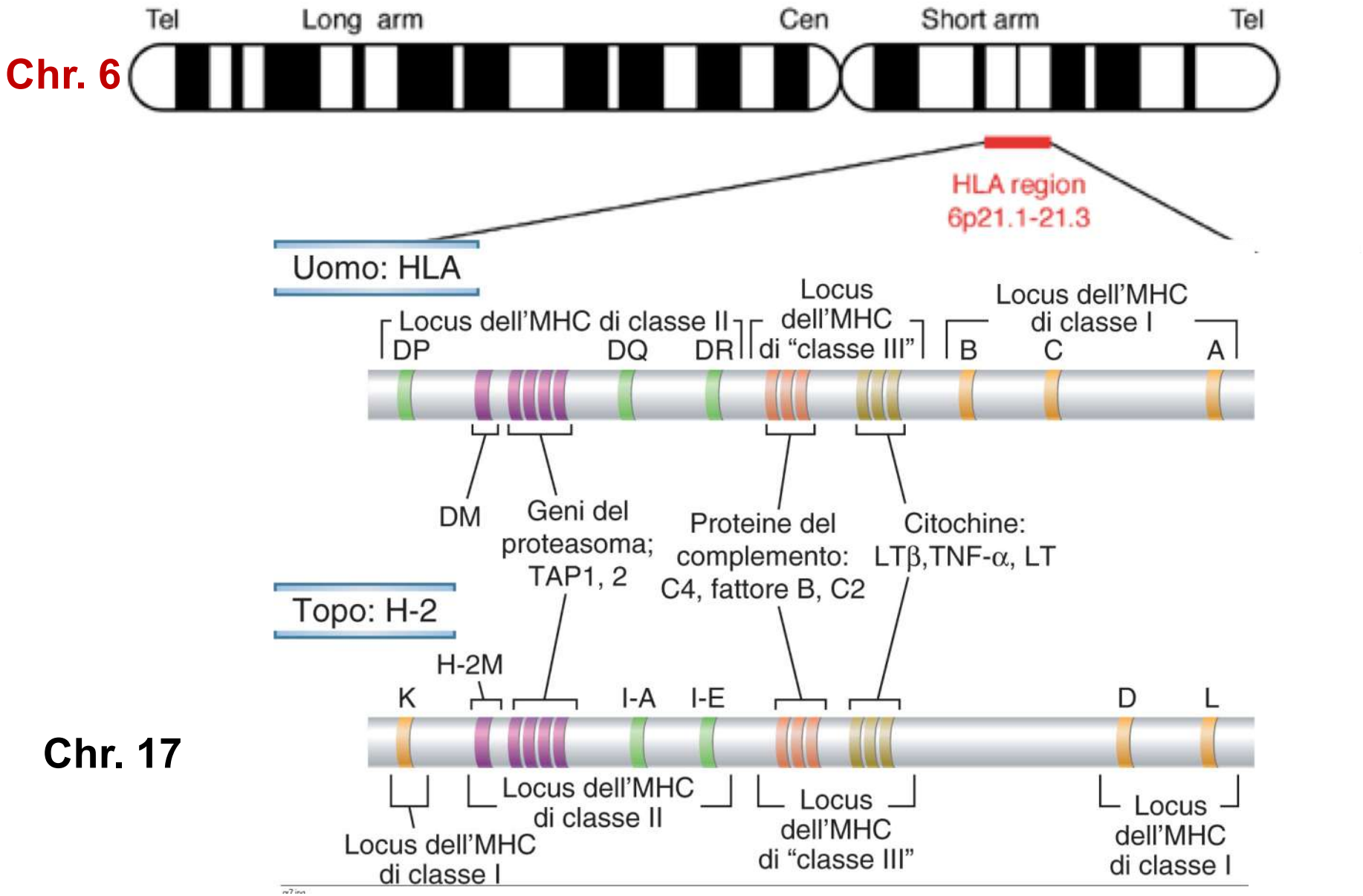
I prodotti di questi geni sono generalmente associati alle risposte immunitarie e coinvolti nell'**attivazione** (receptor signaling pathway, threshold, costimolazione), **proliferazione** e **omeostasi** di cellule dell'immunità adattativa.

Sono componenti importanti dei pathways per la processazione degli antigeni, la disponibilità degli antigeni (autoantigeni) e loro eliminazione, il differenziamento di linfociti, funzioni dell'immunità innata, apoptosi, autofagia, signaling e pathways di citochine, interleuchine, chemochine e interferoni

Associazione dei geni del Complesso Maggiore di Istocompatibilità (MHC) con le patologie autoimmuni



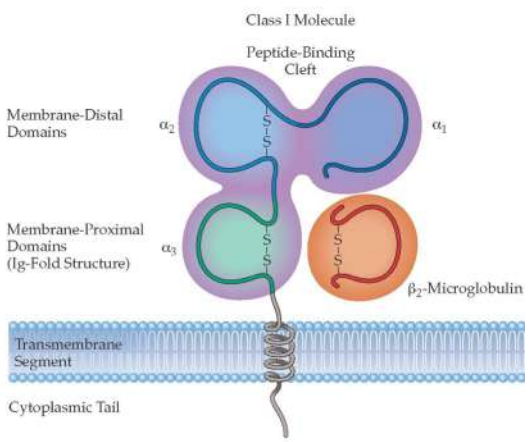
Mappa del complesso maggiore di istocompatibilità (MHC) nell'uomo e nel topo

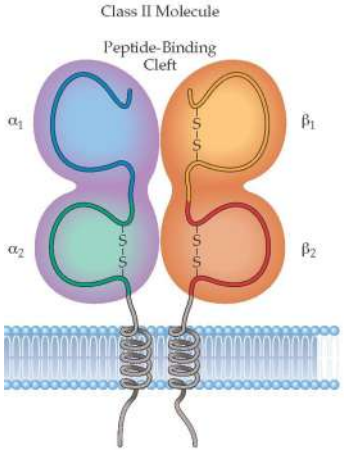


Geni altamente polimorfici

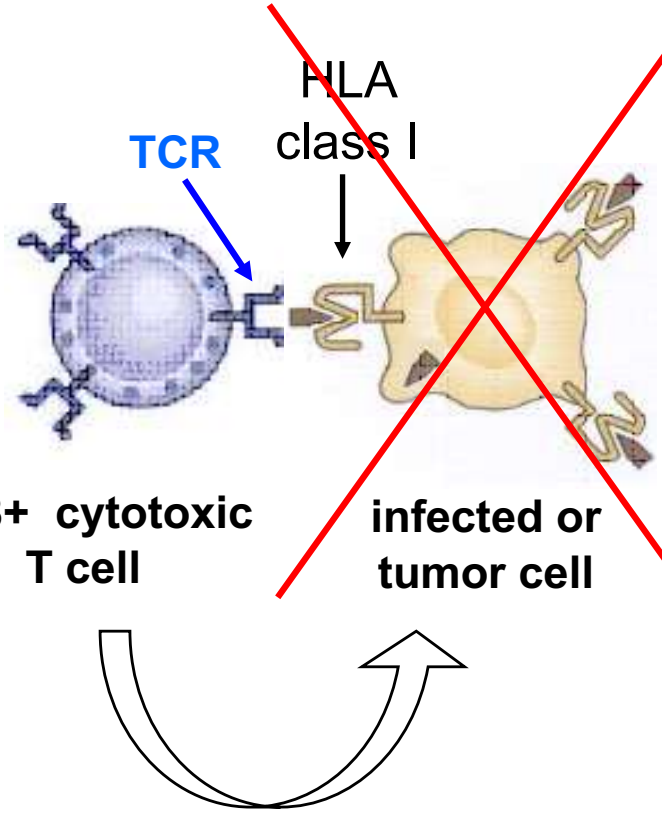
Funzione delle molecole di classe I e II dell'MHC: presentazione dell'antigene

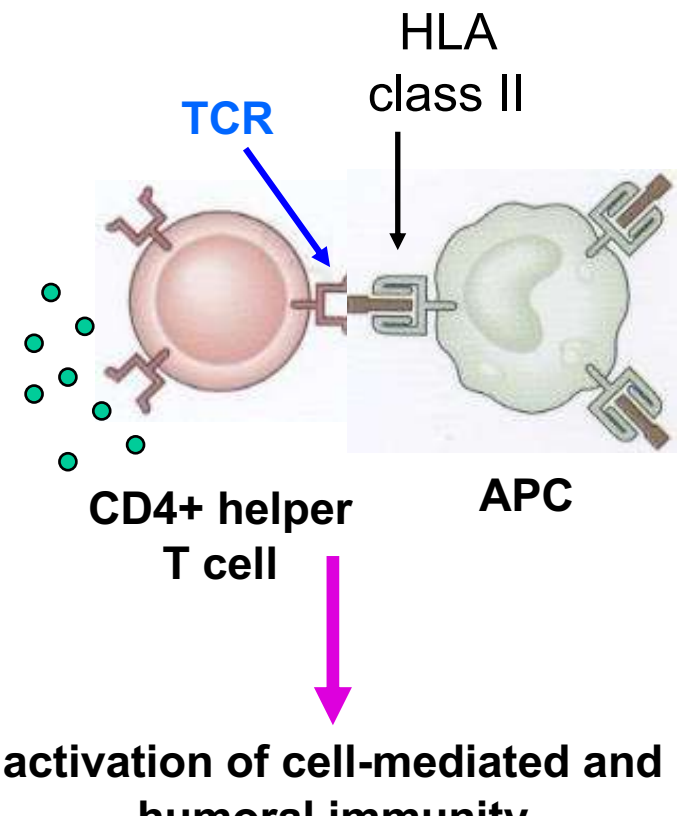
HLA class I



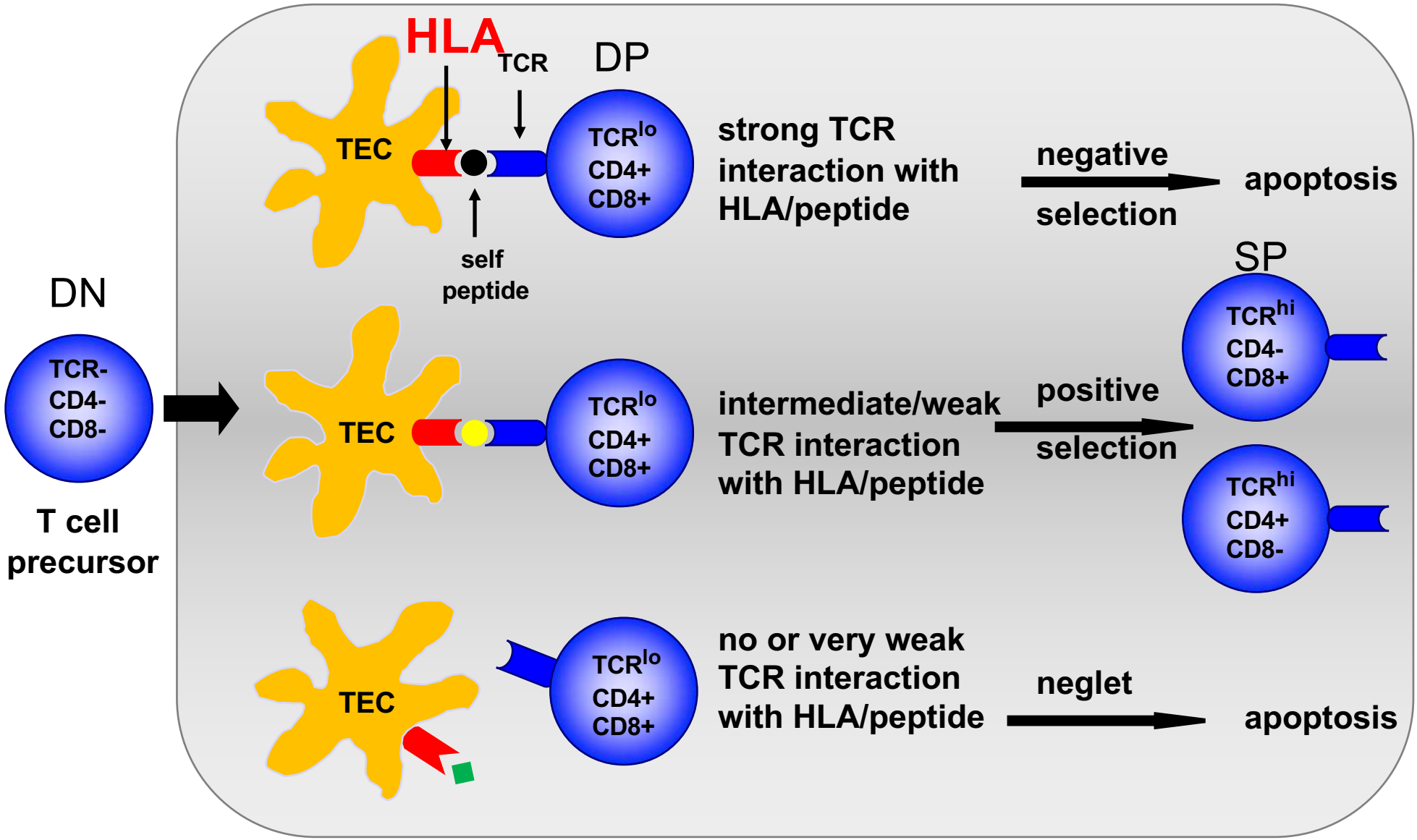


HLA class II





Selezione timica del repertorio dei linfociti T



TEC=thymic epithelial cell
DN=double negative
DP=double positive

Thymus

Associazione del genotipo HLA con la suscettibilità alle malattie autoimmuni

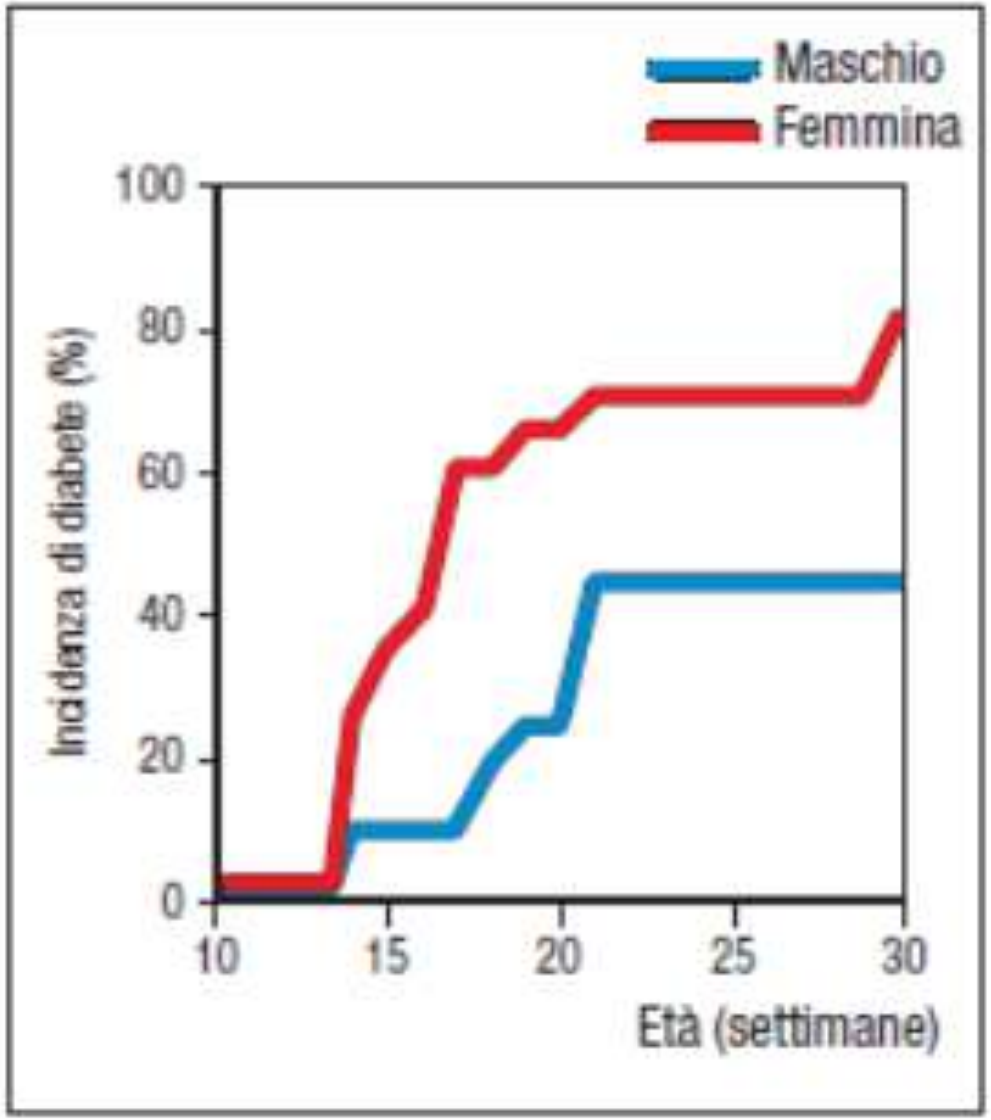
Malattia	Allele HLA	Rischio relativo	Rapporto ♀♂
Spondilite anchilosante	B27	87,4	0,3
Diabete mellito di tipo 1 * (insulino-dipendente)	Eterozigote DR3/DR4	~25	~1
Sindrome di Goodpasture	DR2	15,9	~1
Pemfigo volgare	DR4	14,4	~1
Uveite anteriore acuta	B27	10	<0.5
Psoriasi volgare	CW6	7	~1
Lupus eritematoso sistemico	DR3	5,8	10-20
Malattia di Addison	DR3	5	~13
Sclerosi multipla *	DR2	4,8	10
Artrite reumatoide	DR4	4,2	3
Malattia di Graves	DR3 HLA-B8	3,7	4-5
Tiroidite di Hashimoto	DR5	3,2	4-5
Miastenia grave	DR3	2,5	~1
Diabete di tipo 1	DQ6	0,02	~1

DR2 è protettivo

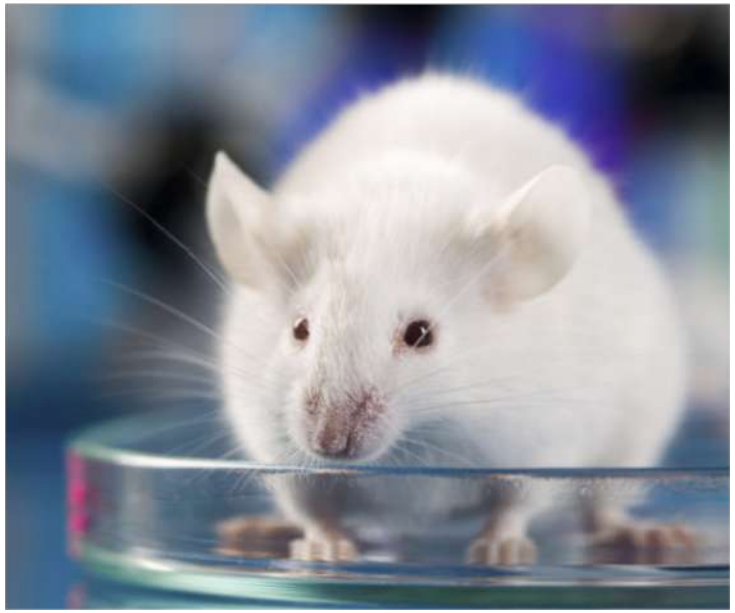
HLA-A2 è protettivo

L'associazione con geni di HLA classe II spesso correla con la produzione di autoanticorpi

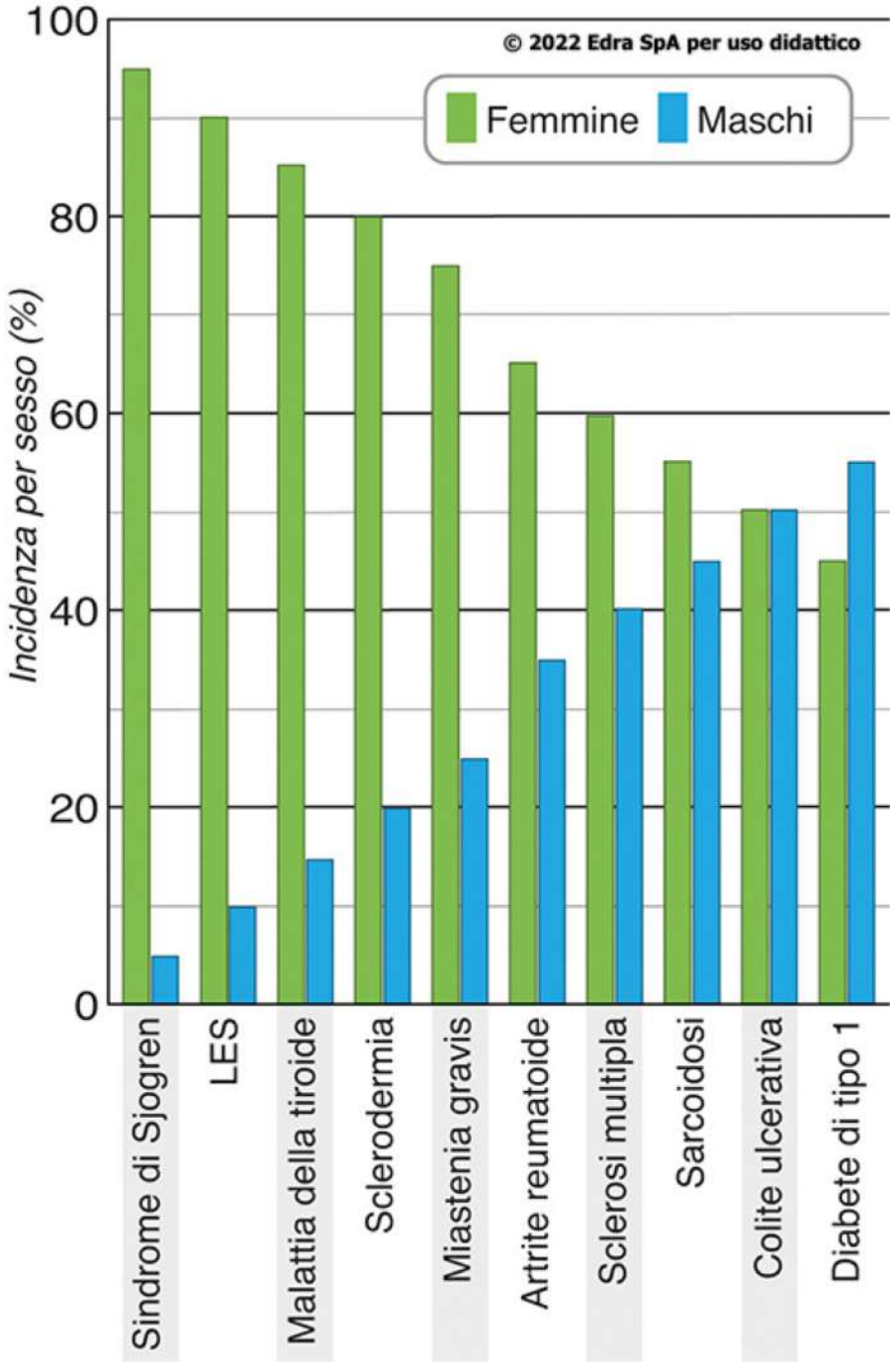
Maggiore predisposizione del sesso femminile allo sviluppo delle patologie autoimmuni



Differenze tra i sessi nell'incidenza delle patologie autoimmuni

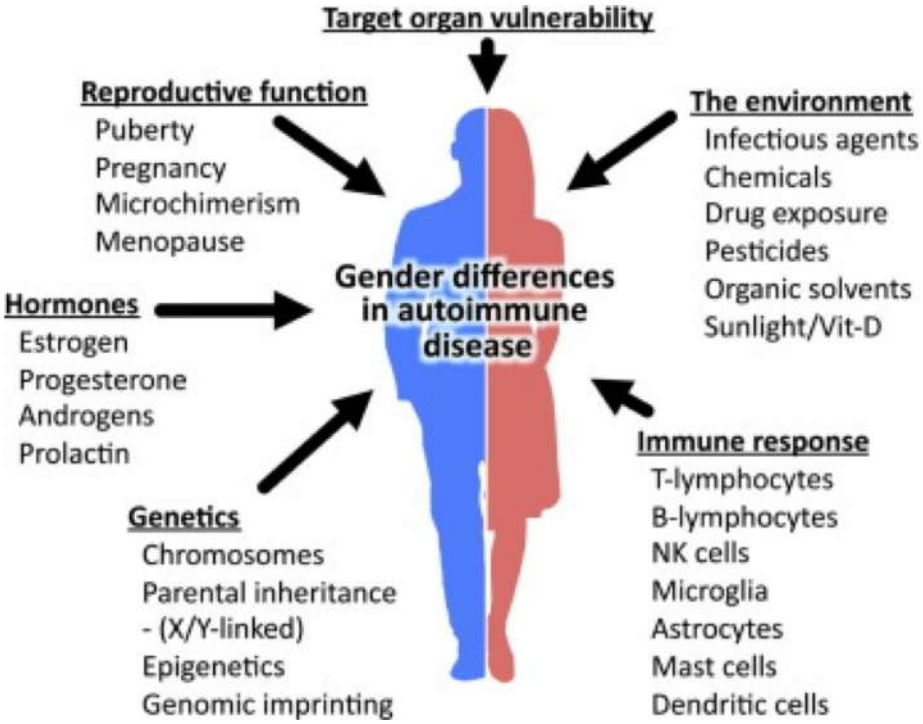


I dati derivano da studi su topi NOD (non-obese diabetic) suscettibili al diabete di tipo 1 (TD1)

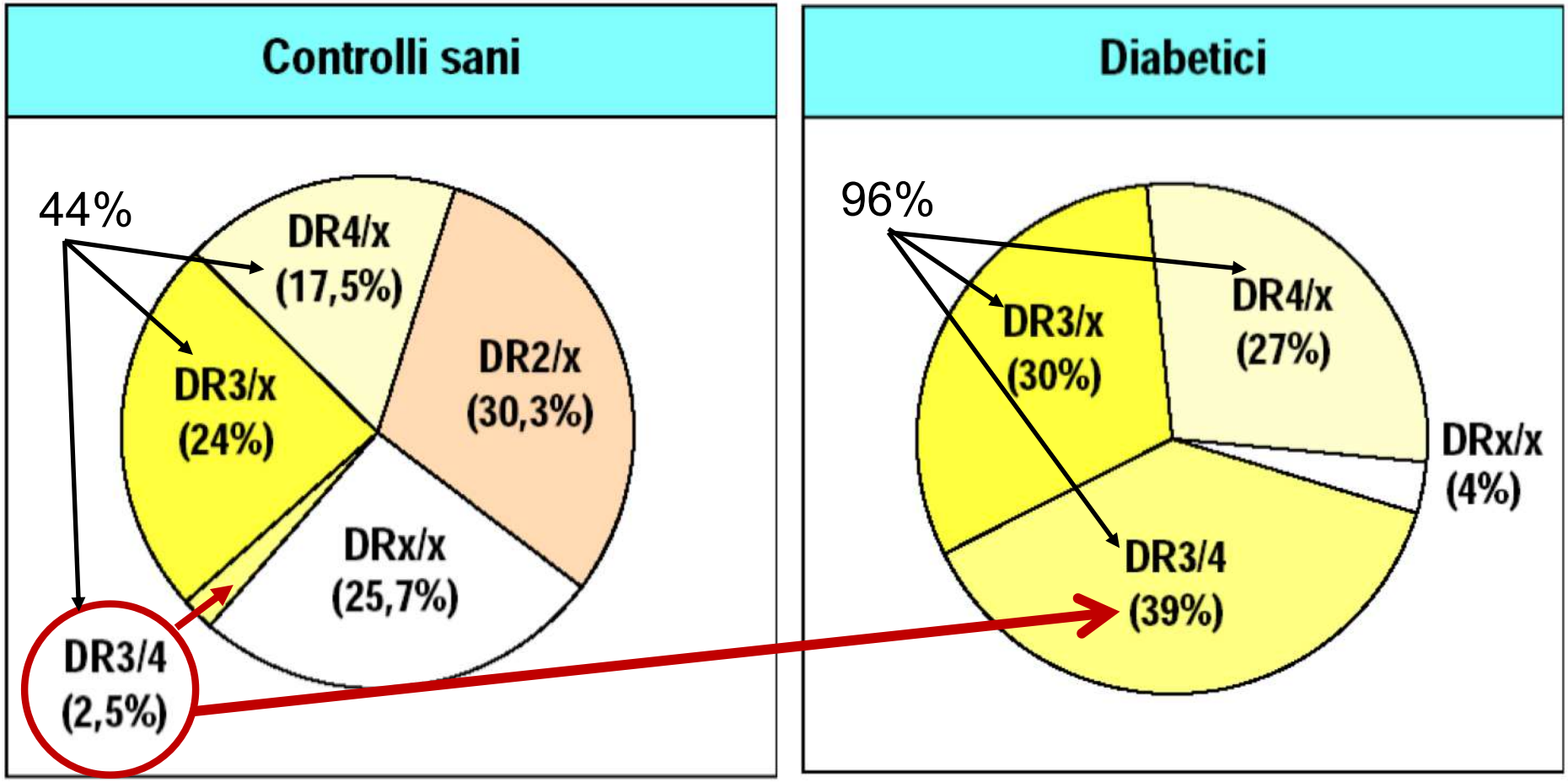


Distribuzione di genere delle principali malattie autoimmuni

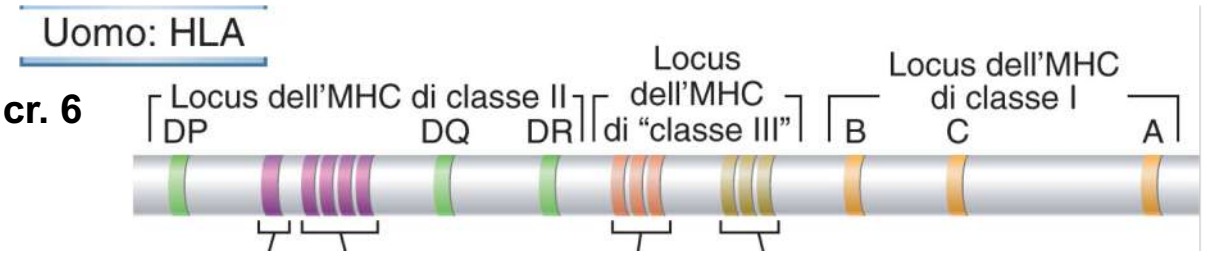
Le percentuali sono approssimazioni basate su dati di incidenza fino all'anno 2000. È inclusa una malattia infiammatoria (sarcoidosi) che non si pensa sia autoimmune. LES = lupus eritematoso sistemico. Sex differences in autoimmune diseases. Nat Immunol. 2001;2:777.



Associazione tra suscettibilità al diabete di tipo I (T1D) e specifici genotipi HLA



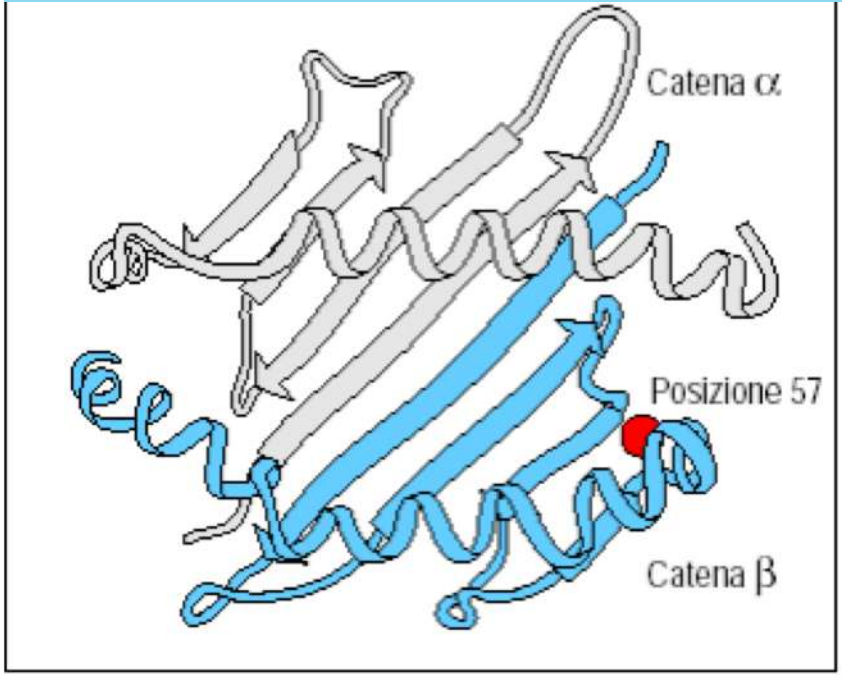
Aplotipi che predispongono all'IDDM: HLA-DQ8-HLA-DR4
HLA-DQ2-HLA-DR3



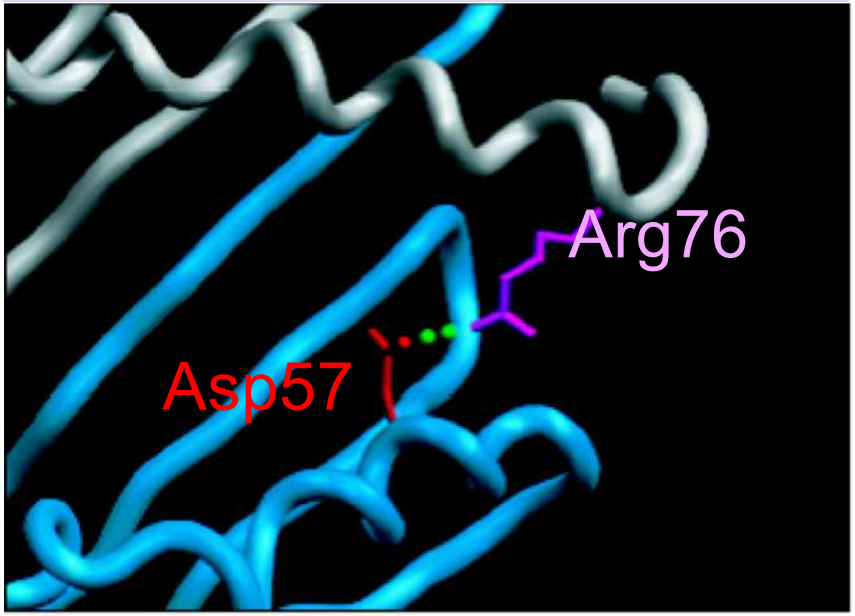
Nell'uomo, la suscettibilità al diabete di tipo 1 (T1D) associa con la variante Val57 (Ser o Ala) nella catena β del DQ

la variante Asp57 è invece protettiva

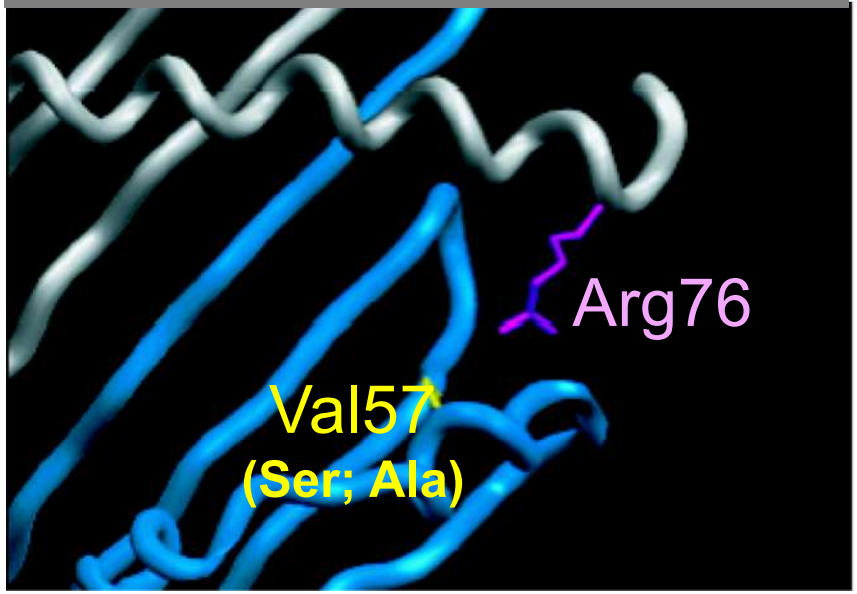
La posizione 57 della catena DQβ controlla la suscettibilità al T1D



Associato con la resistenza al T1D



Associato con la suscettibilità al T1D



I topi NOD (aplotipo H-2g7) esprimono la molecola I-Ag7 (ortologo del HLA-DQ8 e DQ2) che presenta una Ser in posizione 57 nella catena I-A β; (questo è uno dei molteplici fattori di suscettibilità al diabete nei topi NOD)

Associazioni tra geni HLA e malattie autoimmuni

Malattia	Allele HLA associato			Autoantigene	Antigene non self crossreattivo
	Specificita' Sierologica	Sottotipi Molecolari	Aminoacido		
Artrite Reumatoide	DR4	DRB1*0401 DRB1*0404	Arg71 Arg71	Collagene tipo II	
	DR1	DRB1*0101	Arg71	hsp 65	hsp 65 (*Mycobacterium bovis*)
Diabete di tipo I (IDDM)	DR4	DRB1*0402 DRB1*0405		insulina	
	DQw3	DQB1*0301/ DQA1*0302	non-Asp57 Arg52	GAD	P2-C (virus Coxsackie)
	DR3	DRB1*0301			
	DQw2	DQB1*0201/ DQA1*0501	non-Asp57 Arg52	Carbossipeptidasi	
Sclerosi Multipla	DR2 (DR15)	DRB1*1501 DRB1*1502		MBP PLP	DNA polimerasi (virus Herpes simplex) (Epstein-Barr virus) Fosfomannomutasi (*Pseudomonas aeruginosa*) Emoagglutinina (virus influenza)
Spondilite Anchilosante	B27	B*2705 B*2702 B*2704		?	?
Miastenia Grave	B8 DR3			Recettore nicotinico dell'acetilcolina	
Morbo Celiaco	DQw2 DR4	DQB1*0201 DQA1*0501		Gliadina	
Artrite di Lyme	DR4	DRB1*0401		LFA-1	Osp A (*Borrelia burgdorferi*)

GAD, glutammato decarbossilasi; PLP, proteina proteolipidica; MBP, proteina basica della mielina; LFA-1, leukocyte function-associated antigen-1;

aplotipo DRB1*1501, DRB5*0101, DQB1*0602 (aplotipo DR2)

Ipotesi dello "shared epitope" : gli alleli DR associati alla AR condividono la stessa sequenza aminoacidica nella regione 70-74 della catena DRβ

Associazione degli aplotipi HLA alle malattie autoimmuni

Malattia	Allele HLA	Odds Ratio[1]
Artrite reumatoide (positivi per anticorpi anti-CCP)[2]	DRB1, 1 SE allele[3]	4
	DRB1, 2 SE allele	12
Diabete di tipo 1	Aplotipo DRB*0301-DQA1*0501-DQB1*0201	4
	Aplotipo DRB1*0401-DQA1*0301-DQB1*0302	8
	Eterozigosi DRB1*0301/0401	35
Sclerosi multipla	DRB1*1501	3
Lupus eritematoso sistemico	DRB1*0301	2
	DRB1*1501	1,3
Spondilite anchilosante	B*27 (soprattutto B*2705 e *2702)	100
Malattia celiaca	Aplotipo DQA1*0501-DB1*0201	7

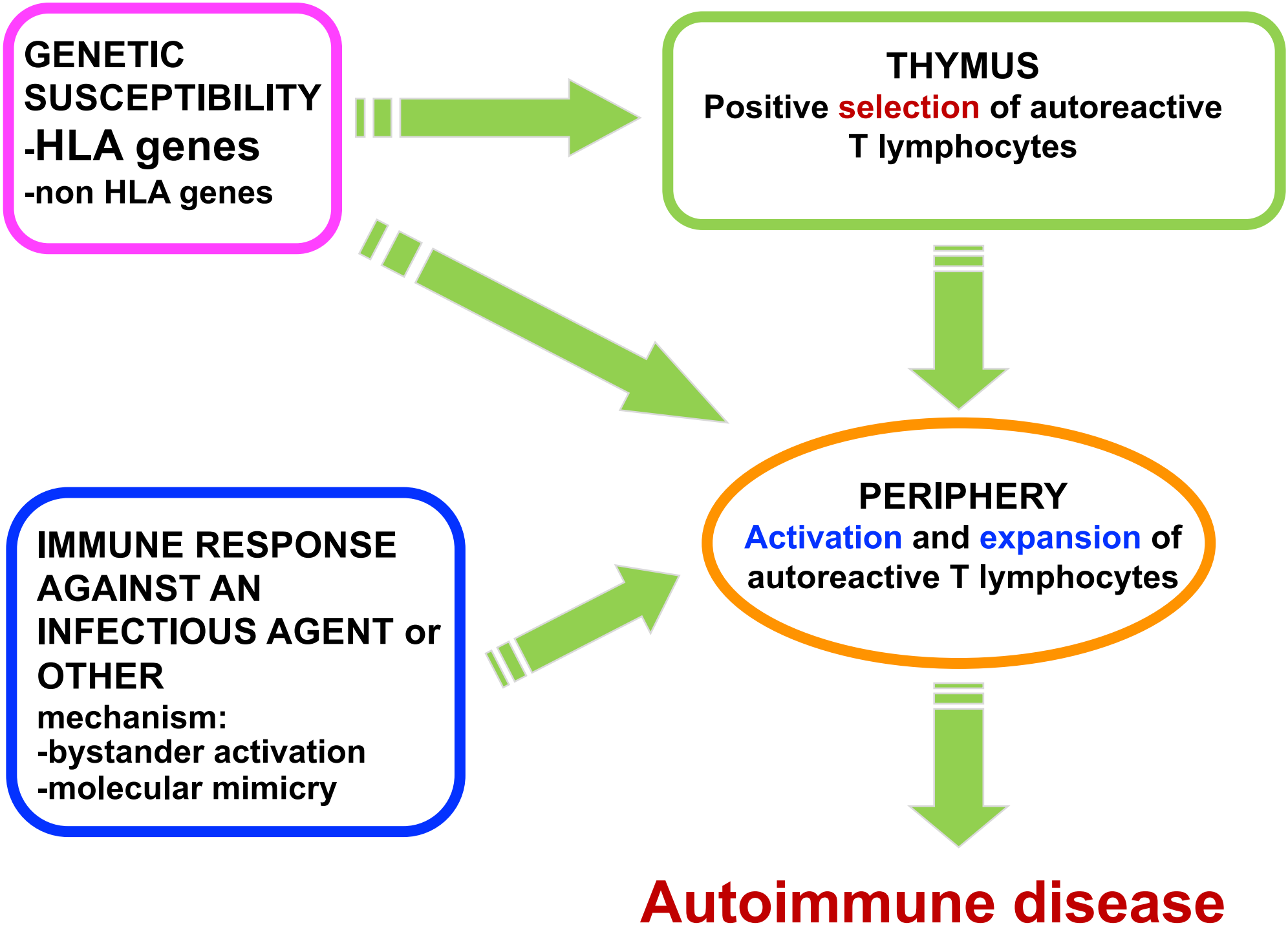
SE=Shared epitope

[1]L'odds ratio rappresenta il valore dell'aumento di rischio di malattia associato all'ereditarietà di alcuni alleli HLA. Dati ottenuti da popolazioni europee.
[2]Anticorpi anti-CCP diretti contro peptidi citrullinati ciclici. Dati ottenuti da pazienti positivi per questi anticorpi nel siero.
[3]SE si riferisce a epitopi condivisi, così chiamati perché gli alleli di suscettibilità mappano in una regione delle proteina DRB1 (posizione 70-74).

Proposed mechanisms for MHC-linked susceptibility to autoimmune diseases

- Disease-associated polymorphisms enable the presentation of **key self-peptide(s)** in the target organ of the disease
- Disease-associated MHC molecules present not only relevant self-peptides, but also **microbial peptides** that enable expansion and activation of relevant self-reactive T cells
- Disease-associated polymorphisms result in poor presentation of critical epitopes and thereby favour **escape from thymic tolerance** mechanisms
- Key polymorphisms exert **effects on the T cell repertoire** important in disease initiation and/or amplification

(Protective MHC molecules select regulatory T cells with specificity for the target organ)


GENETIC SUSCEPTIBILITY
-HLA genes
-non HLA genes
THYMUS
Positive selection of autoreactive T lymphocytes
IMMUNE RESPONSE AGAINST AN INFECTIOUS AGENT or OTHER
mechanism:
-bystander activation
-molecular mimicry
PERIPHERY
Activation and expansion of autoreactive T lymphocytes
Autoimmune disease

Approcci genetici per identificare i geni di suscettibilità alle patologie autoimmuni in modelli animali e nell'uomo

Genetica classica (forward genetics): manipolazione genetica di uno specifico gene (over-espressione per transgenesi; knocking-out o mutagenesi) e si osserva poi il fenotipo indotto

Genetica inversa (reverse genetics): si parte da un ceppo animale che spontaneamente sviluppa la patologia (o da coorti di individui malati rispetto ai sani) e si procede all'analisi di associazione per identificare i loci genici responsabili di quel fenotipo

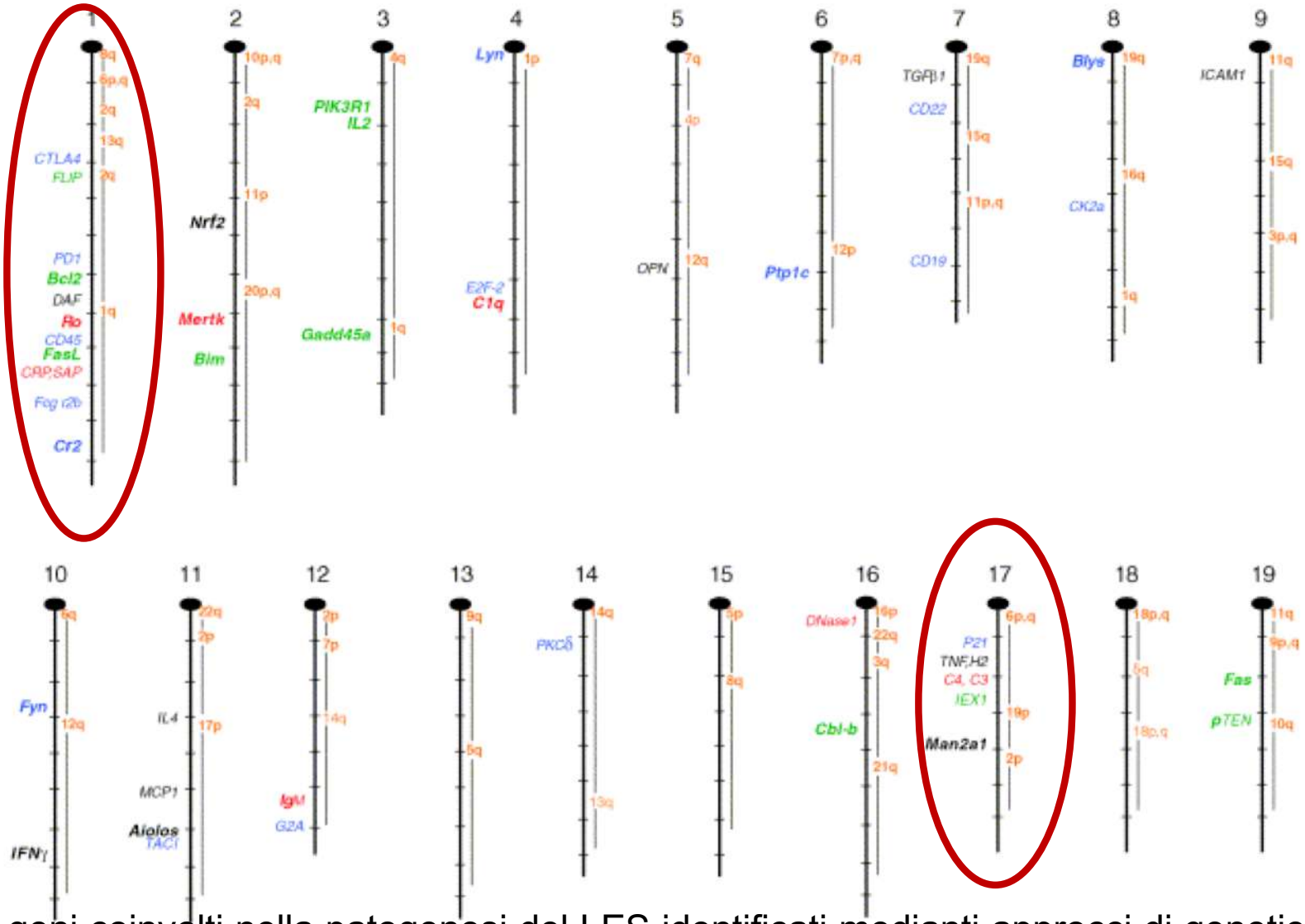
Approcci:

1. Candidate gene association studies (pre-GWAS studies)

2. Family-based linkage analysis (studies)

3. **GWAS**=genome wide association studies; Studi di associazione caso-controllo su tutto il genoma

Analisi genetica del LES mediante forward genetics



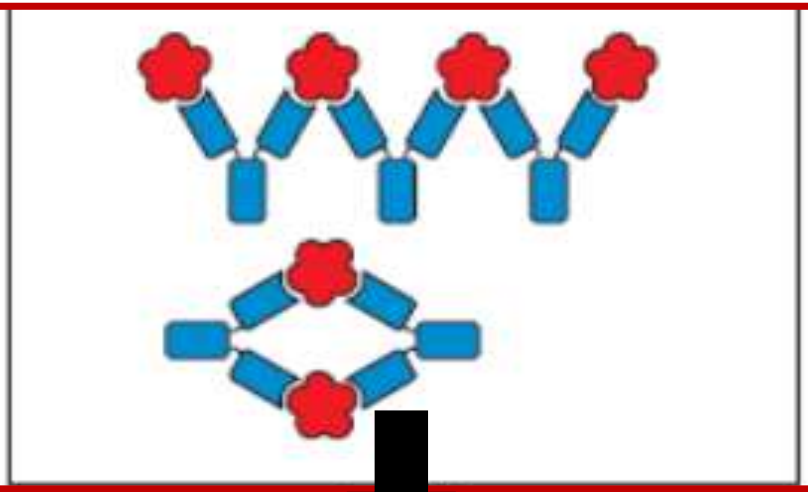
Potenziali geni coinvolti nella patogenesi del LES identificati mediante approcci di genetica classica: in **rosso** quelli coinvolti nella **clearance delle cellule apoptotiche** (C1q; SAP; CRP) in **verde** quelli coinvolti nei **processi apoptotici** (Fas; FasL; Bim; Bcl2) in **blu** quelli che **modulano** segnali per **l'attivazione e l'espansione dei linfociti** (CD19; Lyn; Fyn; CD22; p21, CTLA4; PD1). Sono rappresentati i cromosomi murini (i 19 autosomi) e vicino sulla destra, sono allineati i segmenti cromosomici umani corrispondenti.

Alcuni geni coinvolti nell'eziologia del LES

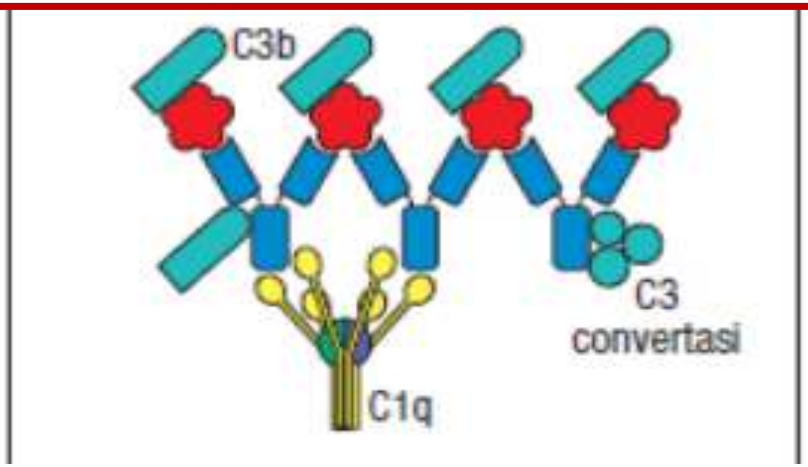
Geni	Ruolo	Deficienza nel LES
Geni codificanti proteine implicate nei meccanismi di eliminazione di molecole derivanti da cellule morte o morenti (possibili fonti di autoantigeni).	Legame ed eliminazione degli autoantigeni e degli immuno-complessi	Proteine complemento: C1q, C1r, e C1s, C4>>C2 IgM sieriche
	Modificazione e digestione del DNA e della cromatina	Componente P amiloide sierica DNase 1
Geni codificanti proteine che regolano la soglia di attivazione, la tolleranza linfocitaria, l'omeostasi e l'apoptosi	Soglia per l'attivazione dei linfociti	Lyn SHP-1 CD22 FcγRIIB
	Delezione dei linfociti autoreattivi	Fas and Fas-ligando p21 inibitore del ciclo cellulare
	Costimolazione nei linfociti	CTLA4 PD-1 BAFF
Geni che codificano per proteine coinvolte nei pathways di citochine tra cui gli **interferoni di tipo I**		

Meccanismo di eliminazione degli immunocomplessi dal circolo (difettivo nel LES)

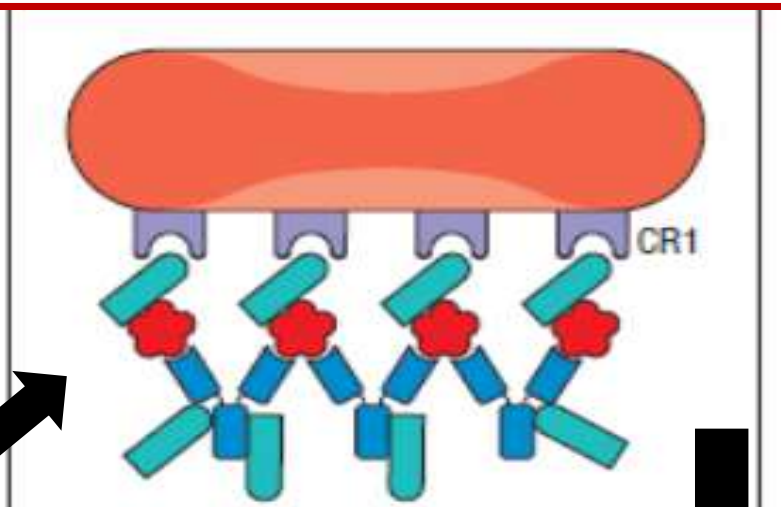
Piccoli complessi antigene/anticorpo si formano in circolo ed attivano il complemento



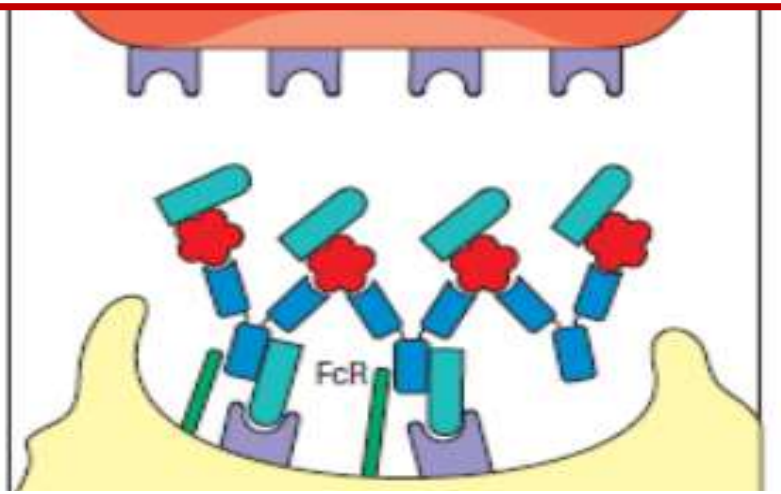
L'attivazione del complemento porta al deposito di molte molecole di C3b sull'immunocomplesso



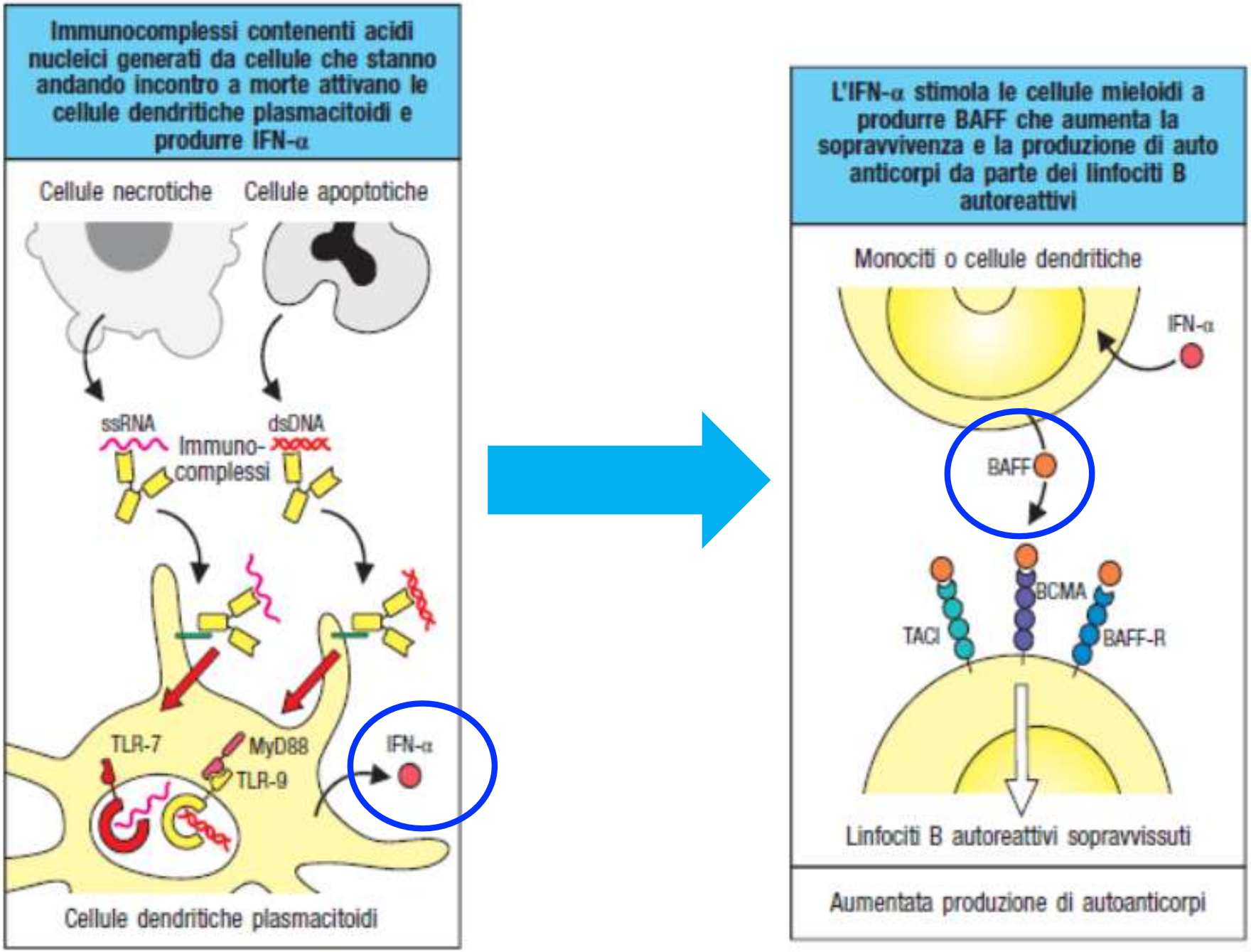
il recettore CR1 sugli eritrociti lega gli immunocomplessi attraverso il C3b



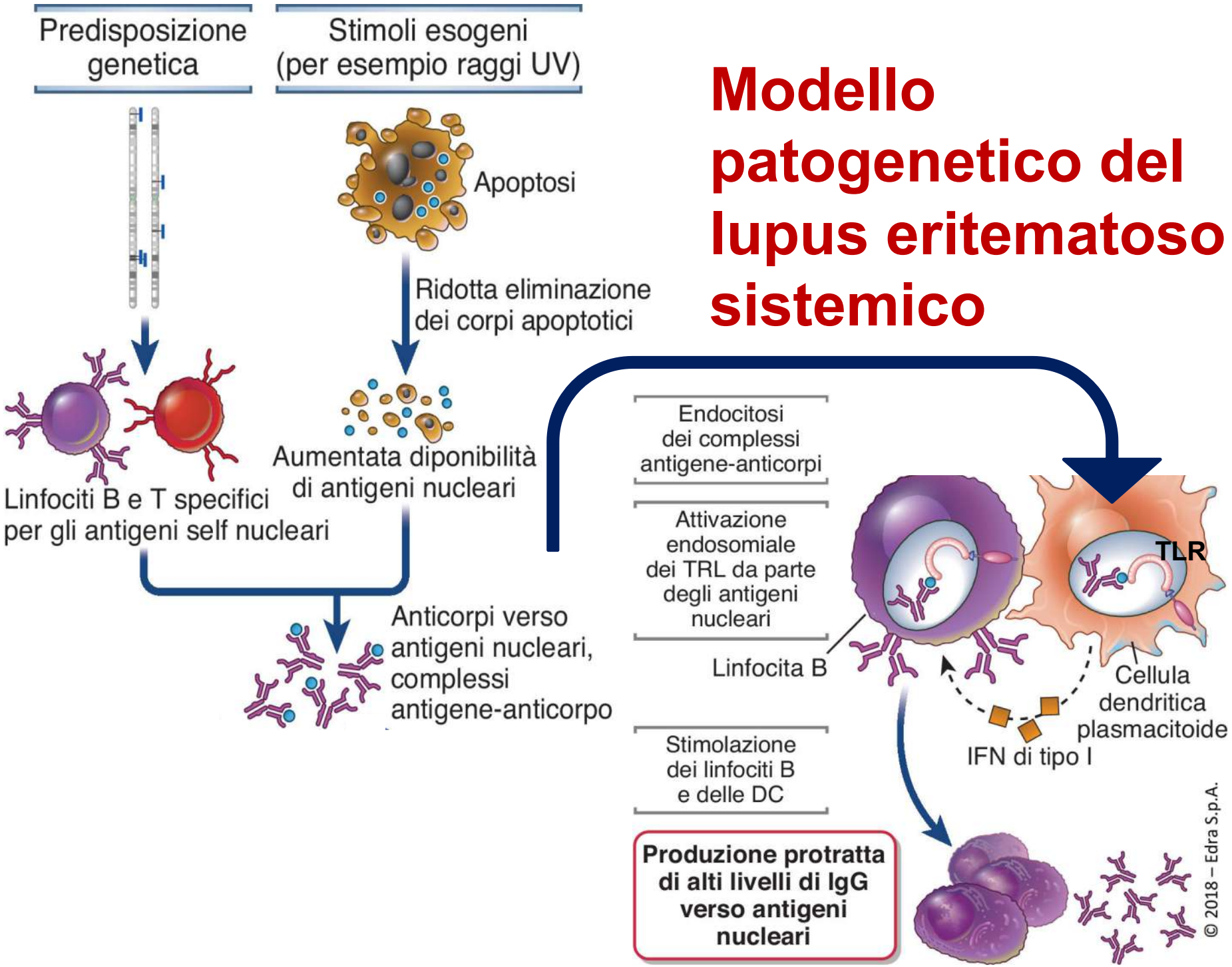
Nella milza e nel fegato, le cellule fagocitiche rimuovono gli immunocomplessi dalla superficie degli eritrociti

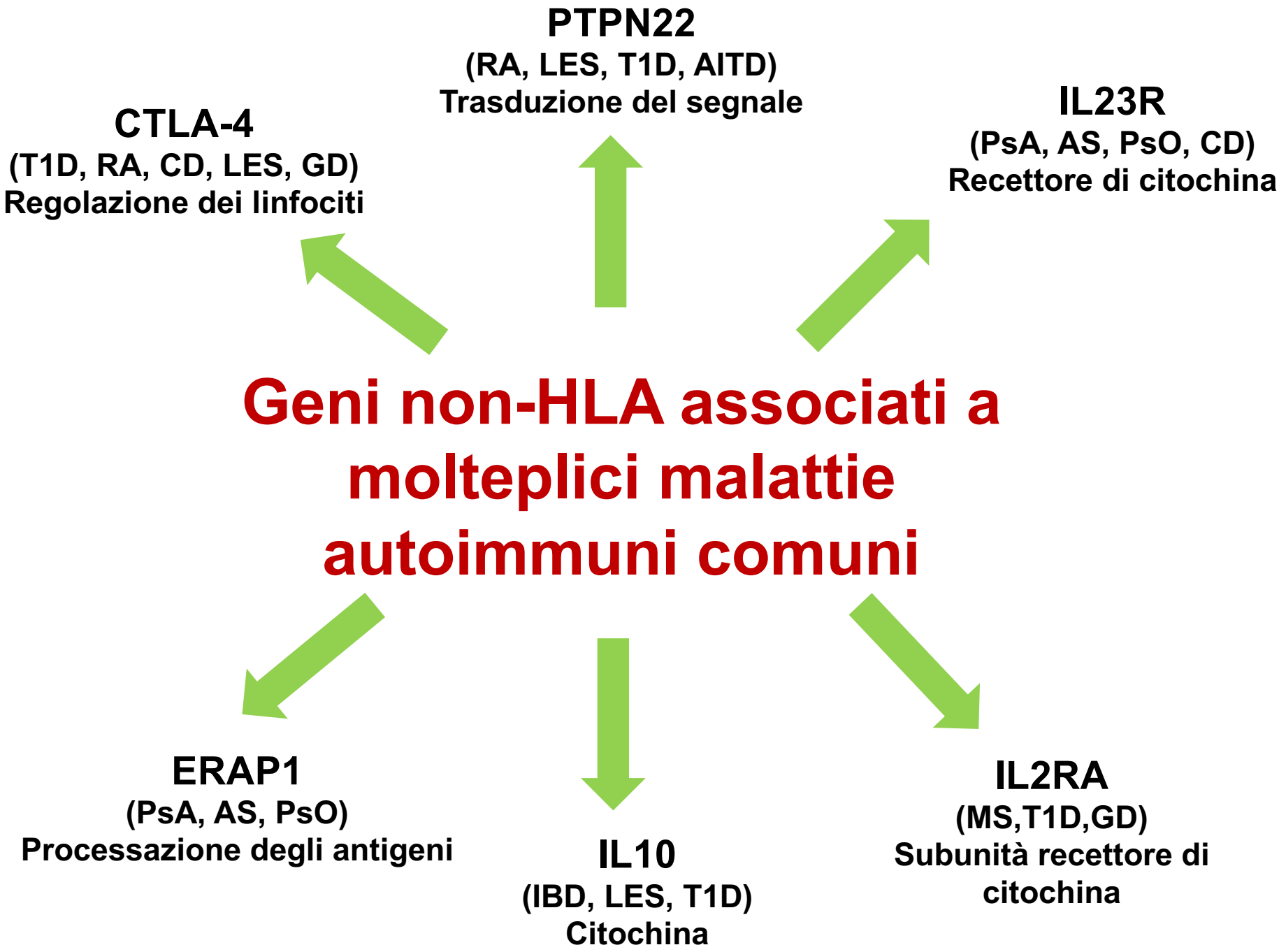


Rimozione difettiva di immunocomplessi contenenti acidi nucleici: implicazioni per il LES



Modello patogenetico del lupus eritematoso sistemico




PTPN22
(RA, LES, T1D, AITD)
Trasduzione del segnale
CTLA-4
(T1D, RA, CD, LES, GD)
Regolazione dei linfociti
IL23R
(PsA, AS, PsO, CD)
Recettore di citochina
Geni non-HLA associati a molteplici malattie autoimmuni comuni
ERAP1
(PsA, AS, PsO)
Processazione degli antigeni
IL10
(IBD, LES, T1D)
Citochina
IL2RA
(MS,T1D,GD)
Subunità recettore di citochina

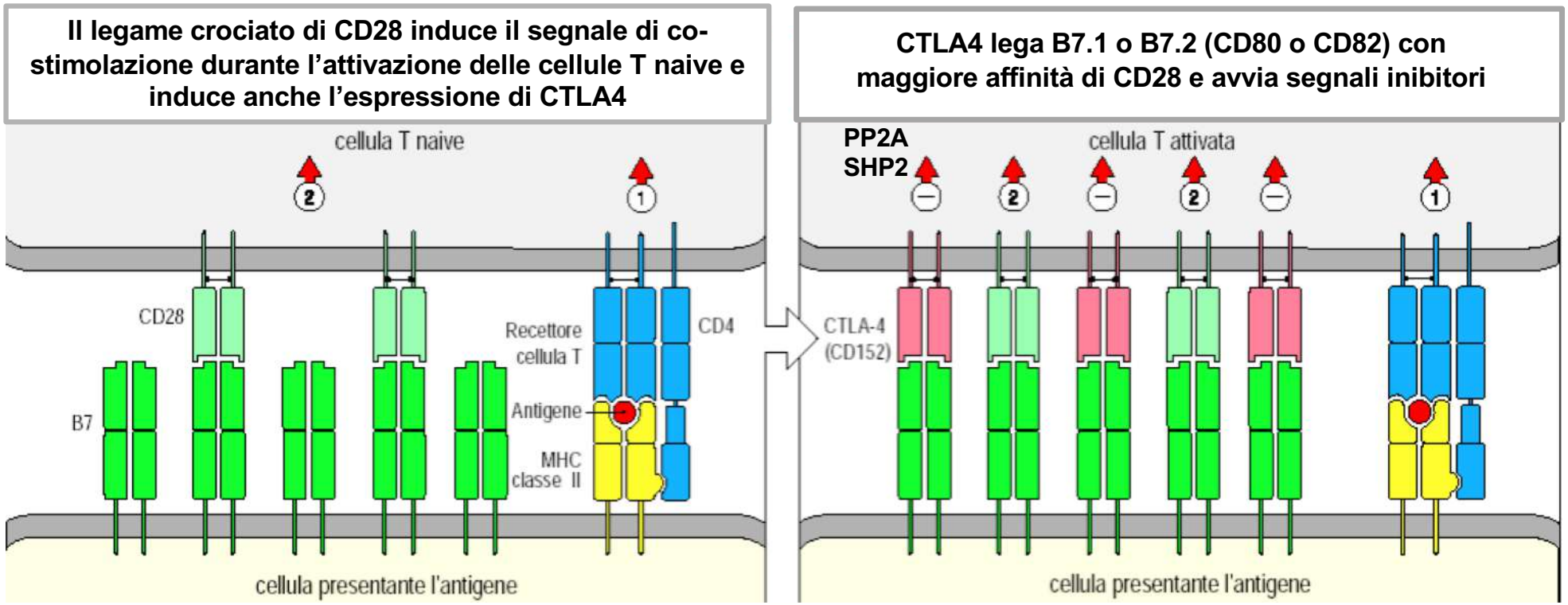
CTLA4: gene di suscettibilità per molte patologie autoimmuni che agisce da recettore inibitorio dei linfociti T e da mediatore di soppressione

CTLA4 (cytolytic T lymphocyte-associated antigen) mappa nella regione 2q33

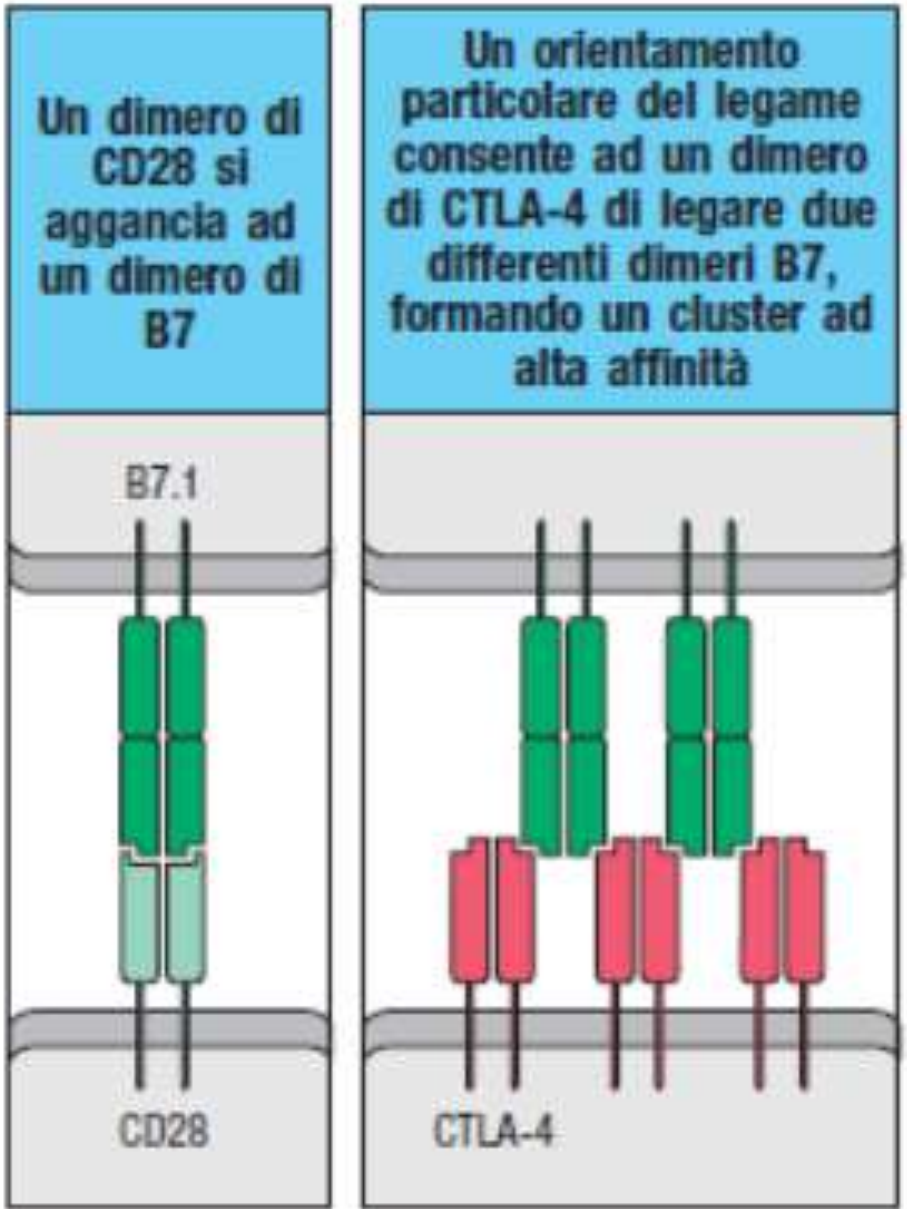
Numerosi studi genetici hanno mostrano associazioni di varianti alleliche di CTLA4 (SNPs e microsatelliti) con patologie autoimmuni: **T1D, morbo di Graves, RA, MS primariamente progressiva, celiachia, morbo di Addison, LES ed altre**

Nel 2014 è stata identificata nell'uomo una patologia a trasmissione mendeliana causata da mutazioni in eterozigosi di CTLA4 (CHAI= CTLA4 haploinsufficiency with autoimmune infiltration)

Topi knockout per CTLA4 sviluppano una sindrome linfoproliferativa fatale



Esistono tre isoforme di CTLA4: **"full-length", forma solubile e "ligando indipendente" (identificata esclusivamente nel topo)**



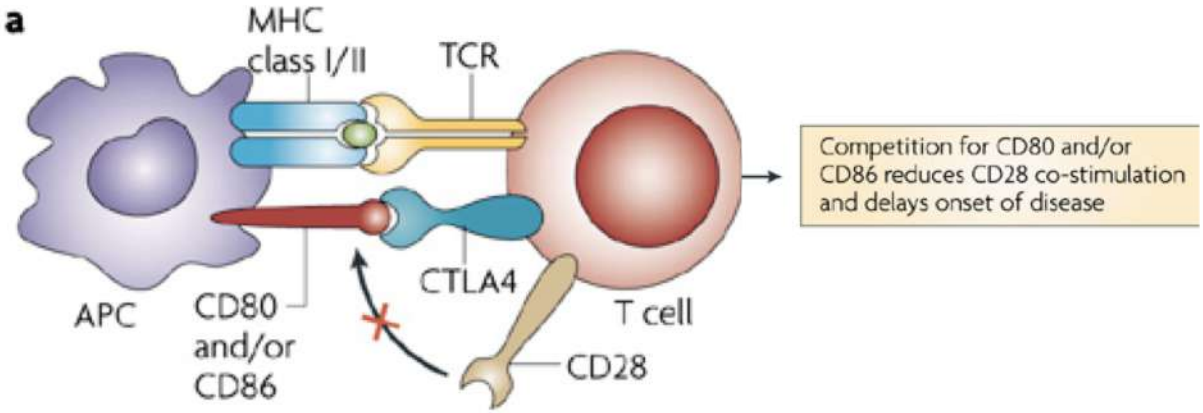
CTLA-4 (immune checkpoint) ha un'affinità maggiore per B7 rispetto al CD28 e lo impegna in un legame con orientamento multivalente

CTLA4 an Essential Immune Checkpoint for T-Cell Activation

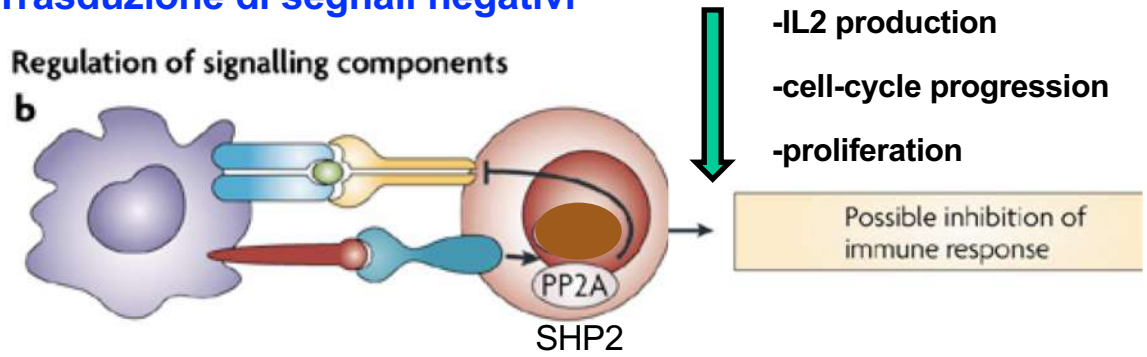
Possibili modelli funzionali di CTLA4

meccanismi basati su ‘’cell-intrinsic factors”

Competizione



Trasduzione di segnali negativi



PP2A= protein phosphatase 2A

Several possible mechanisms by which CTLA4 may inhibit T-cell responses. While some are more speculative than others, there is some experimental evidence to support all these possibilities. Accordingly, polymorphisms in CTLA4 genes may affect only one or many of these mechanisms.

(A) CTLA4 competes with CD28 for ligands. In this model, the higher affinity and avidity of CTLA4 for the ligands CD80 and CD86 can potentially prevent or at least diminish CD28 signaling, because insufficient ligand binds to CD28. This model does not presume a CTLA4 'signal'. Polymorphisms causing unusually high levels or stabilized CTLA4 surface expression could influence via this mechanism. **Likewise, high levels of soluble CTLA4 may be inhibitory**.

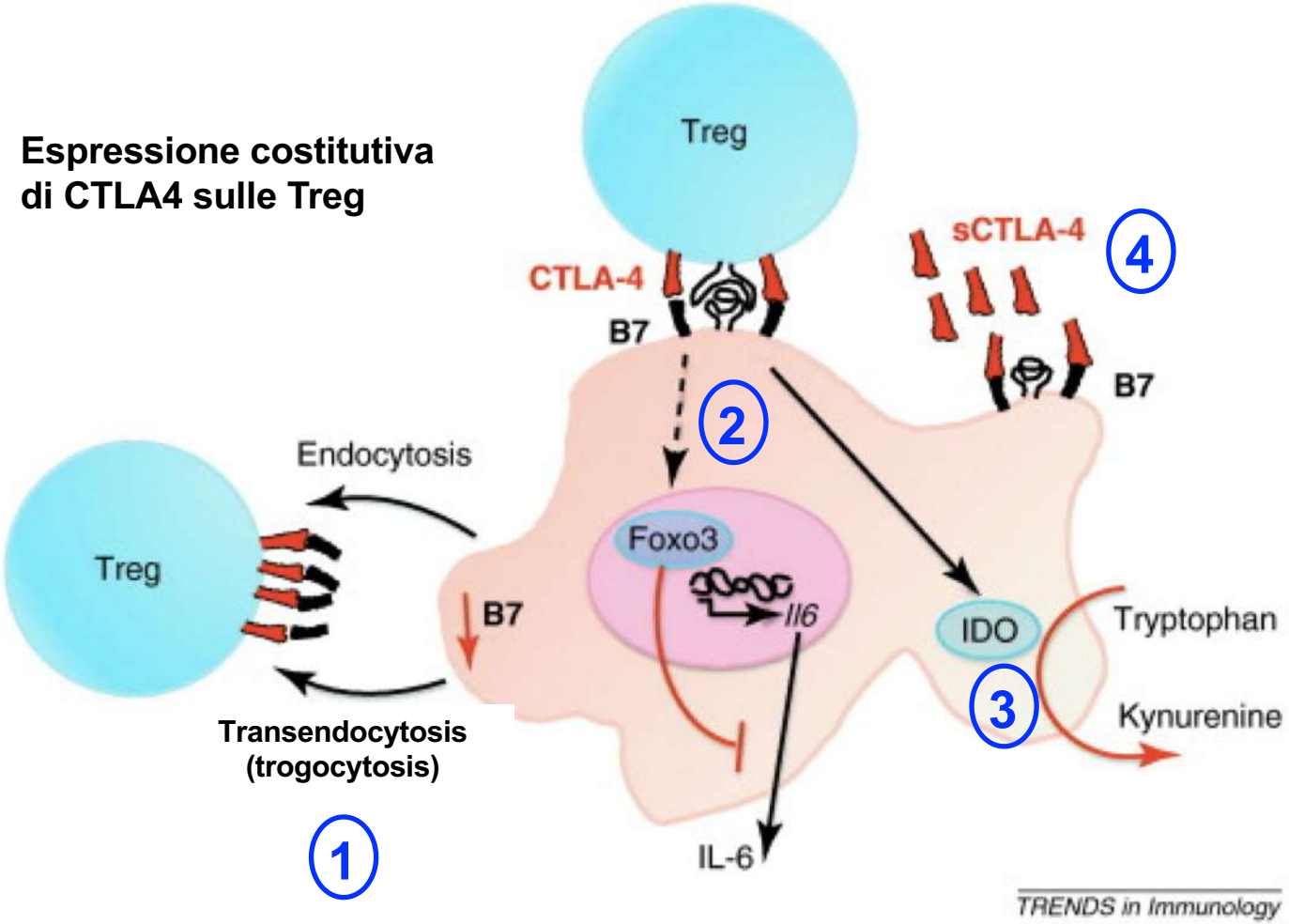
(B) CTLA4 generates inhibitory signals. In this model, CTLA4 is thought to recruit inhibitory molecules such as phosphatases, leading to diminished CD28/T-cell receptor signals. Thus, the negative signal from CTLA4 would override positive signals required for activation. Note that this is a 'cell intrinsic' model, and each cell affected by CTLA4 function must express CTLA4.

Potenziali modelli funzionali di CTLA4

Meccanismi basati su “cell-extrinsic factors”

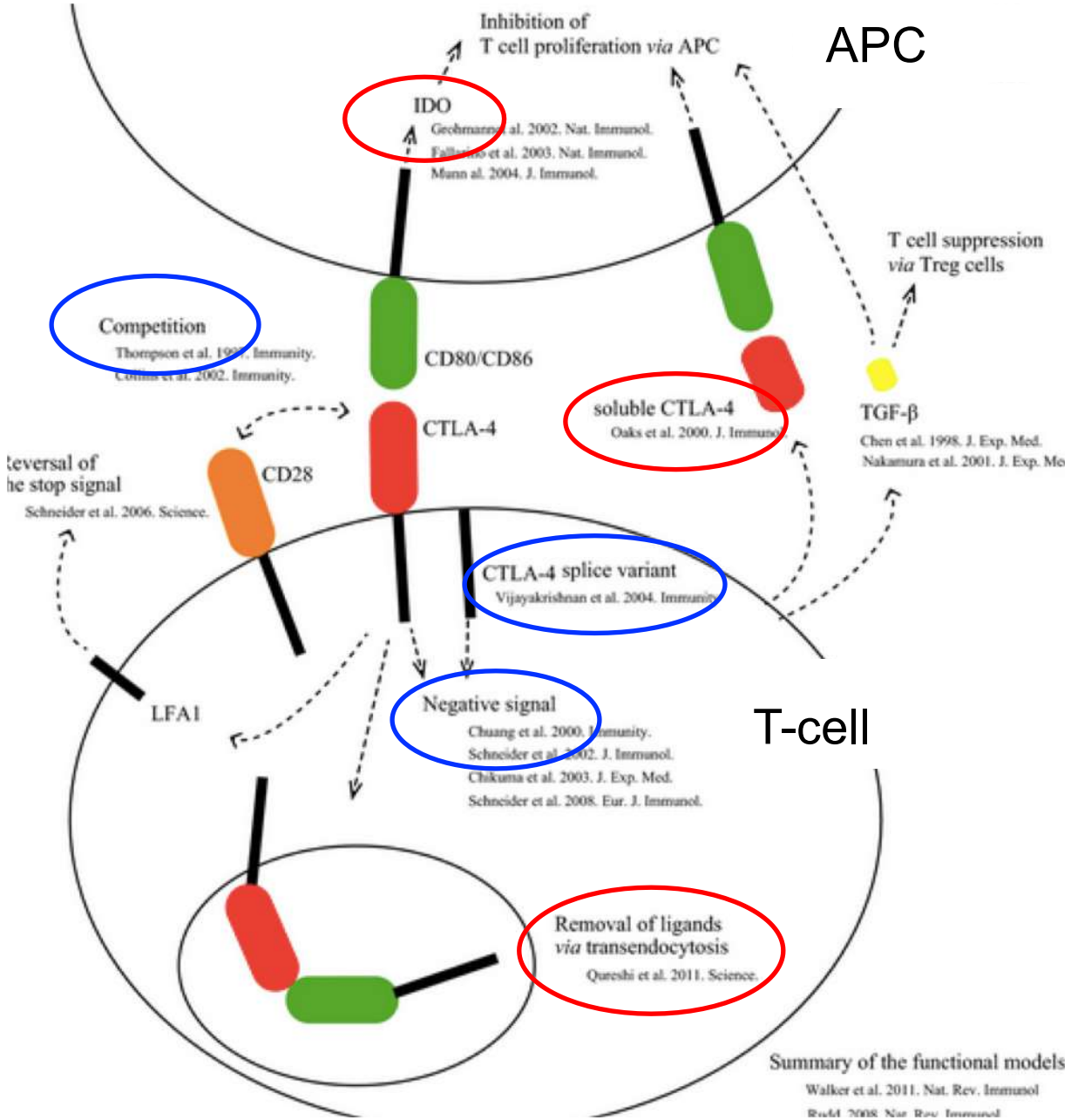
Azione sulle APC

Espressione costitutiva di CTLA4 sulle Treg



CTLA-4 can modulate APC function by several different mechanisms.
CTLA-4-expressing T cells can decrease expression of the B7 (CD80 and CD86) molecules on APCs, and thereby modulate APC capacity to prime naïve T cells.
-For example, CTLA-4 can mediate trogocytosis of CTLA-4-bound CD80 and CD86 molecules from the APC to the Treg cell membrane or the Treg cells can endocytose them.
-CTLA-4 ligation can also affect the transcriptional machinery of APCs by inducing nuclear translocation of Foxo3, which downregulates IL-6 expression of DCs.
-Furthermore, it has been shown that CTLA-4 ligation induces IDO-dependent catabolism of tryptophan to the immuno-suppressive kynurenine in DCs, which might lead to cell death. Furthermore, secreted soluble CTLA-4 might block CD80 and CD86, thereby preventing proper co-stimulation via CD28 on T cells.

The functional model of CTLA-4



The main functions of CTLA-4 are divided into **cell-intrinsic factors** and **cell-extrinsic factors.**

In the intrinsic models, CTLA-4 delivers a negative signal to the CTLA-4 expressing T cell after T cell activation. A splice variant of CTLA-4 can transmit the inhibitory signal.

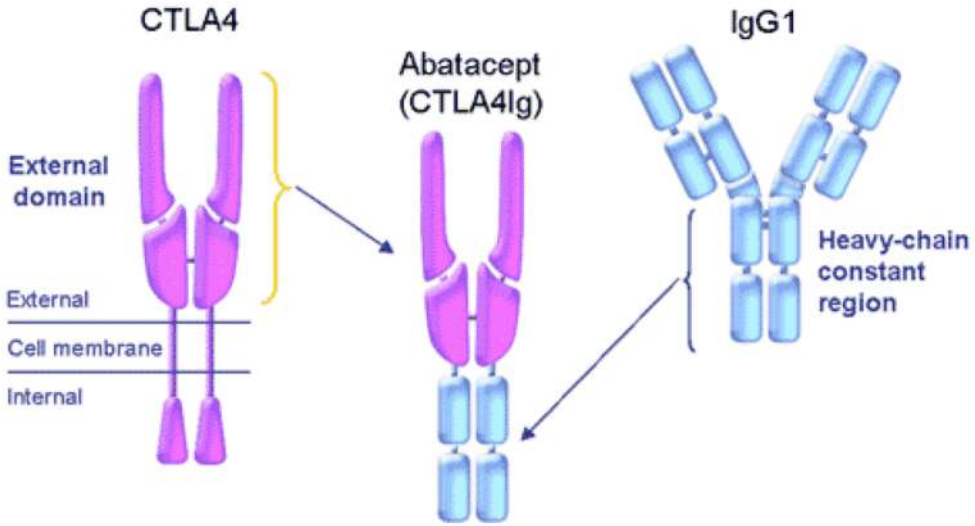
The competition with CD28 in binding to CD80 or CD86 leads to the inhibition of T cell activation. These ligands are physically removed by CTLA-4-mediated trans-endocytosis.

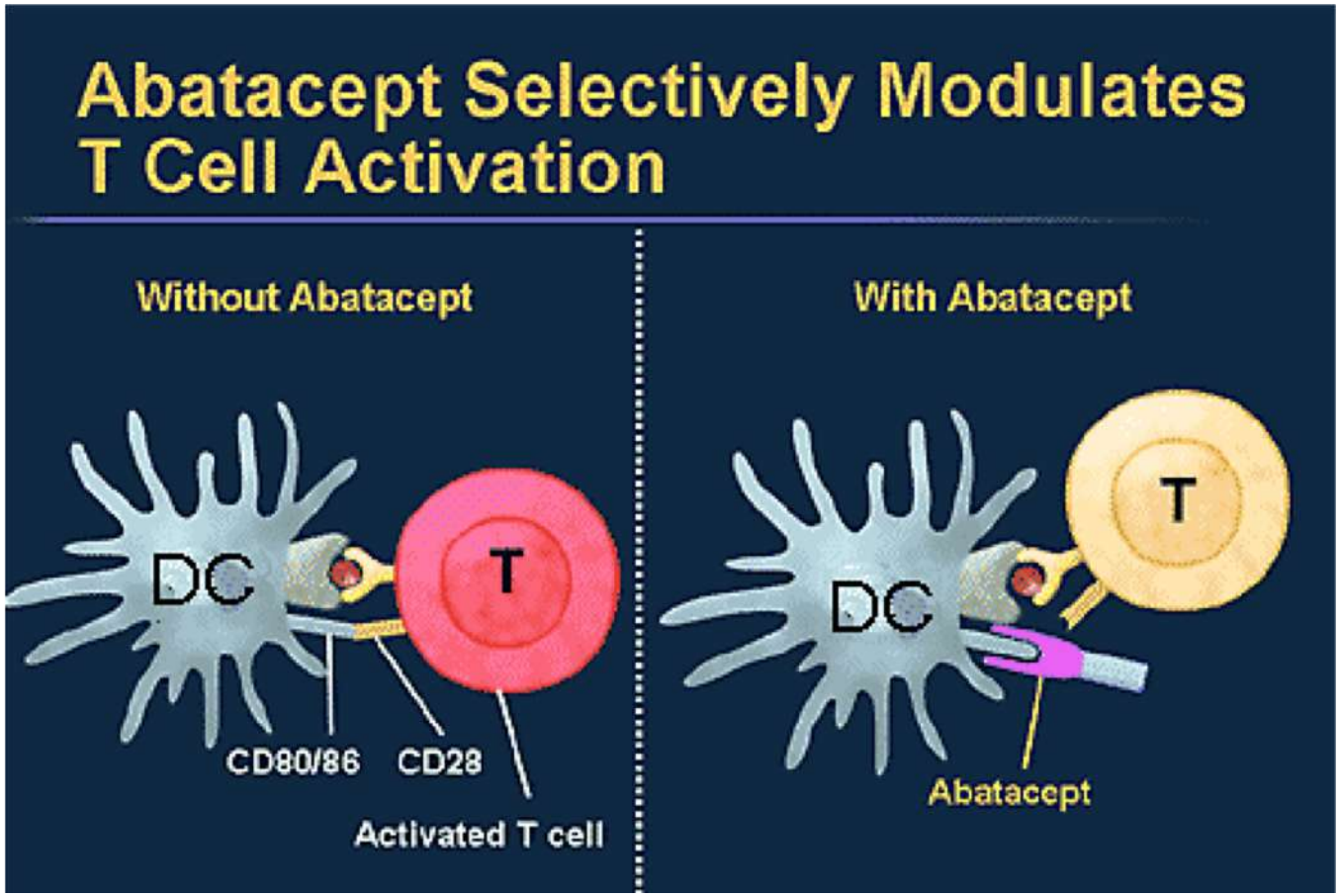
CTLA-4 has the ability of the reverse stop signal, controlling the adhesion and the motility between APCs and T cells. In the extrinsic models, T cell proliferation is inhibited via APCs modulated following the ligation of CTLA-4 and TGF-β. Treg cells stimulated upon TGF-β also suppress T cell activation. APC, antigen-presenting cells; Treg cells, regulatory T cells; TGF-β, transforming growth factor-β; LFA1, lymphocyte function-associated antigen 1;

IDO, indoleamine 2,3-dioxygenase

ABATACEPT

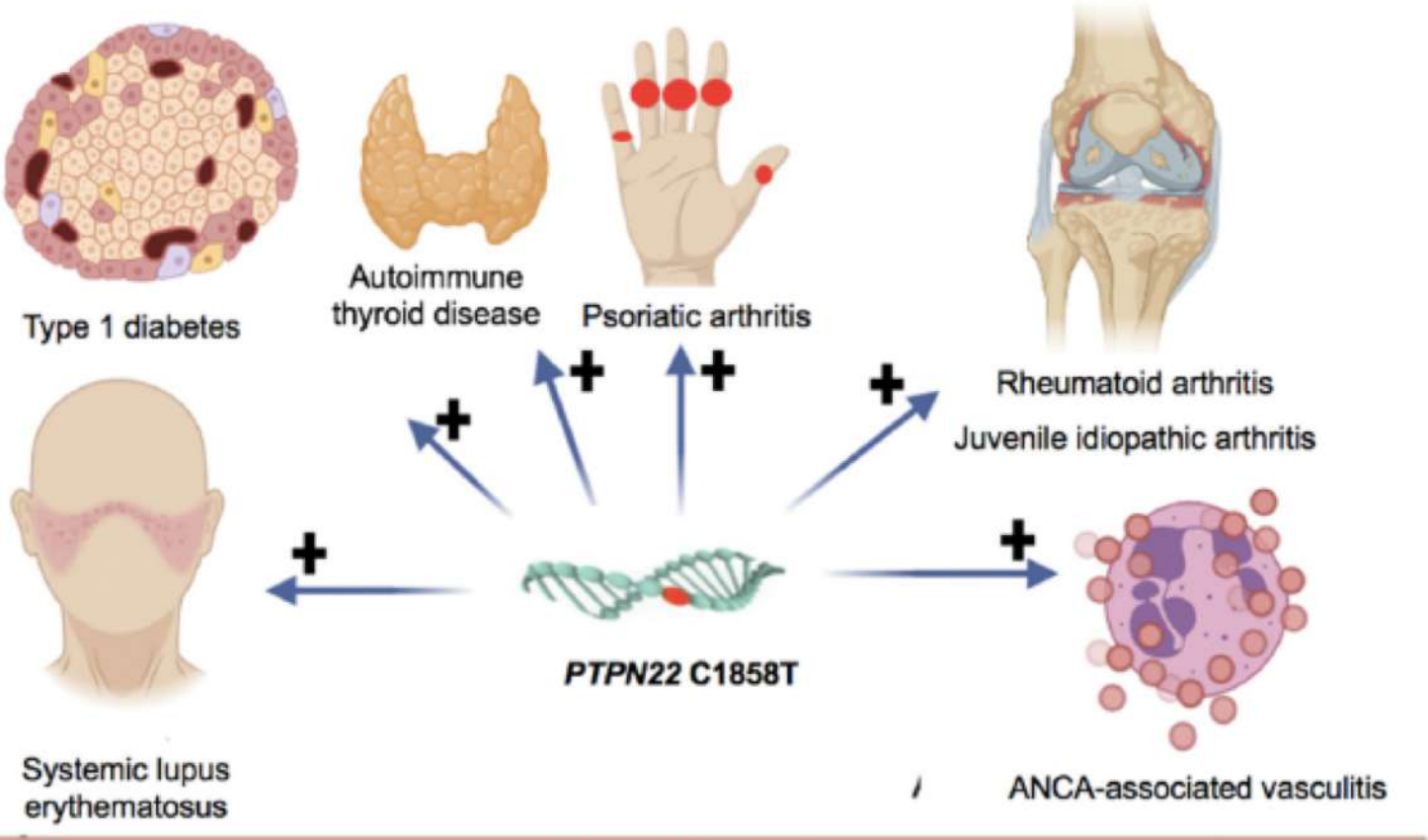
(farmaco biologico usato nella cura della RA;
CTLA4 solubile-Ig
fusione del dominio extracellulare di CTLA4 con Fc di IgG1)





PTPN22: regolatore multifunzionale dell'immuno-signaling è associato a molte patologie autoimmuni

Patologie associate: T1D; RA, LES; morbo di Graves; tiroidite di Hashimoto; vitiligine; artrite psoriasica; morbo di Addison



Not associated with:

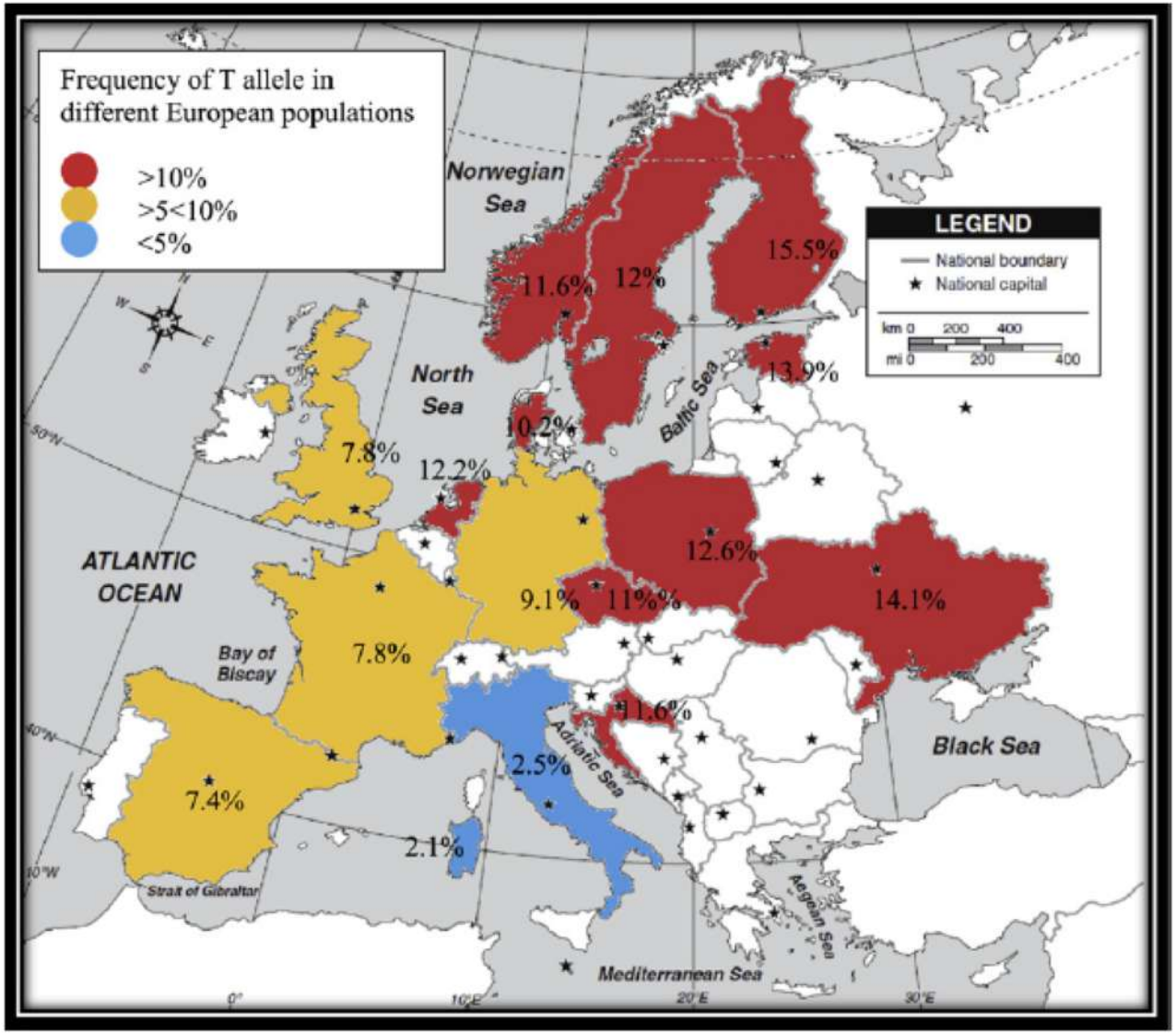
Multiple sclerosis	Primary sclerosing cholangitis
Pemphigus vulgaris	Acute anterior uveitis
Behçet's disease	Primary biliary cholangitis

PTPN22: regolatore multifunzionale dell'immuno-signaling è associato a molte patologie autoimmuni

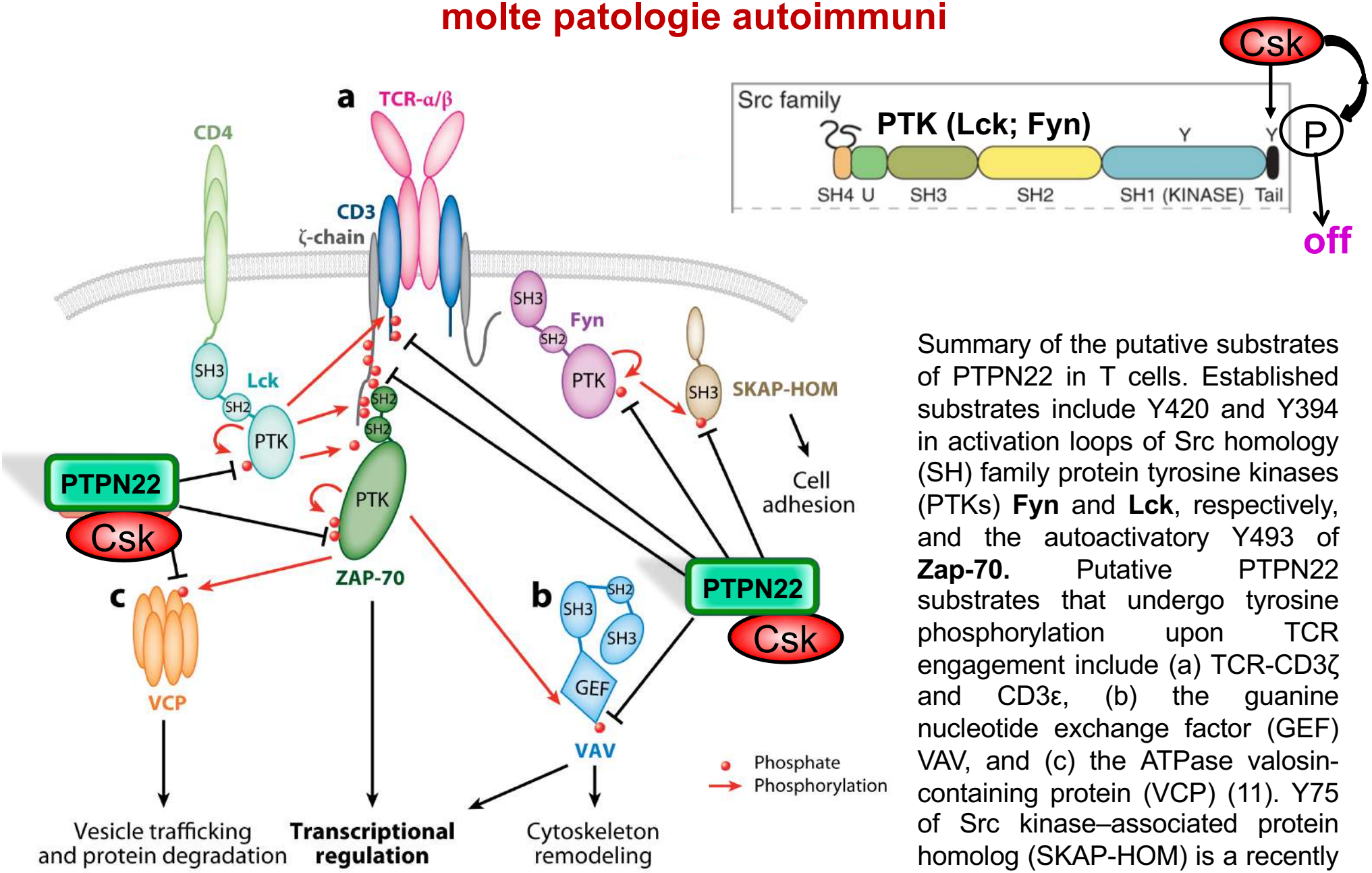
Questo gene (cr.1) codifica per **LYP** (807aa; lymphoid protein tyrosine phosphatase) una **tirosin fosfatasi** coinvolta nel signaling di molti pathways dei linfociti T e B, cellule mieloidi svolgendo una funzione chiave nello sviluppo e attivazione, nell'induzione della tolleranza, difesa da parte dell'immunità innata e nella immuno-regolazione.

In diverse popolazioni, è stata dimostrata l'associazione della variante allelica **T1858 (SNP C1858T)** con diverse patologie autoimmuni che determina in **LYP** la **sostituzione R620W.** Il residuo aminoacidico 620 è compreso in un dominio ricco in proline che interagisce con il dominio SH3 di CSK e con TRAF3.

Frequenza dell'allele T che codifica per la variante di rischio W620 nella popolazione europea

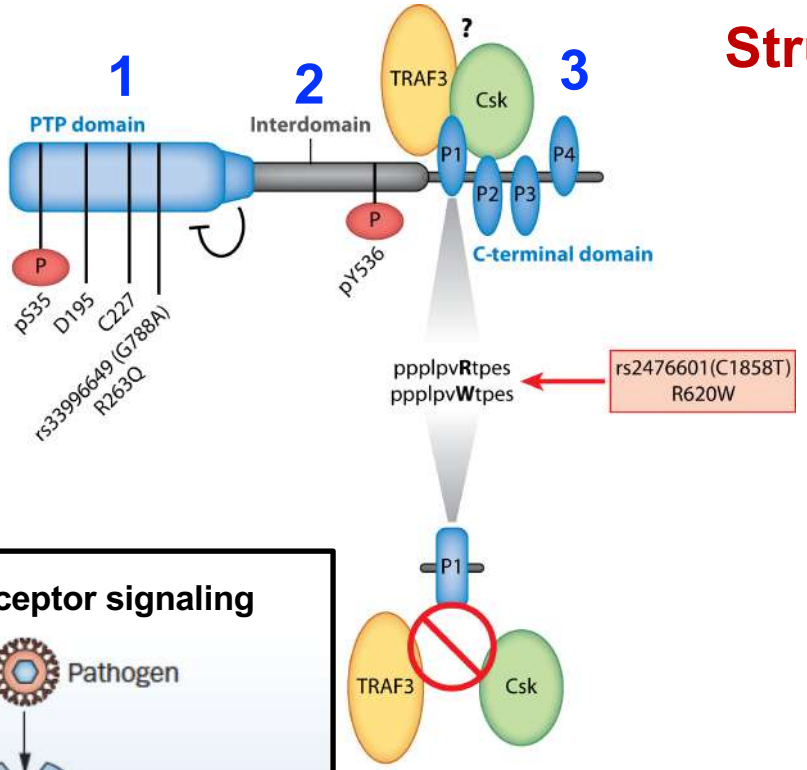


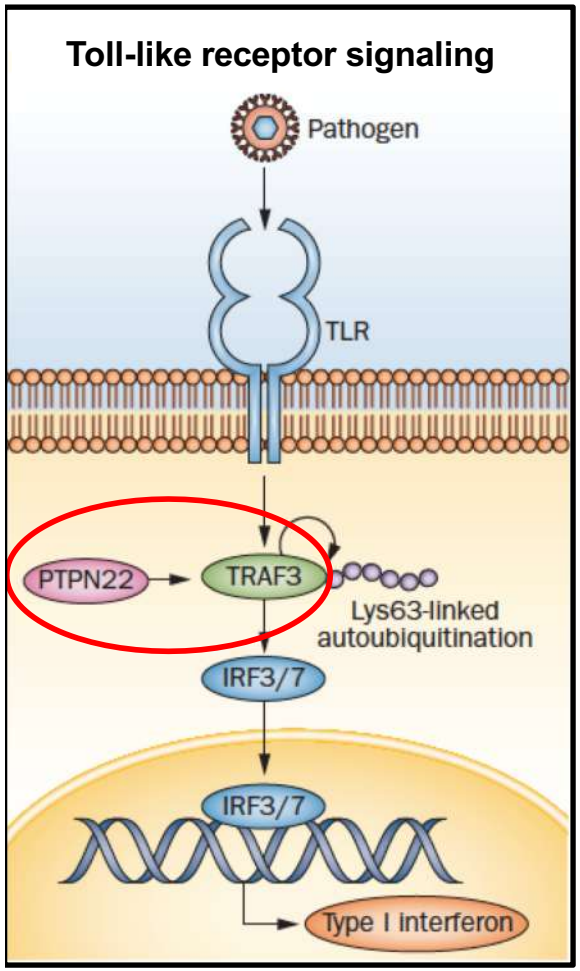
PTPN22 regolatore multifunzionale dell'immuno-signaling è associato a molte patologie autoimmuni

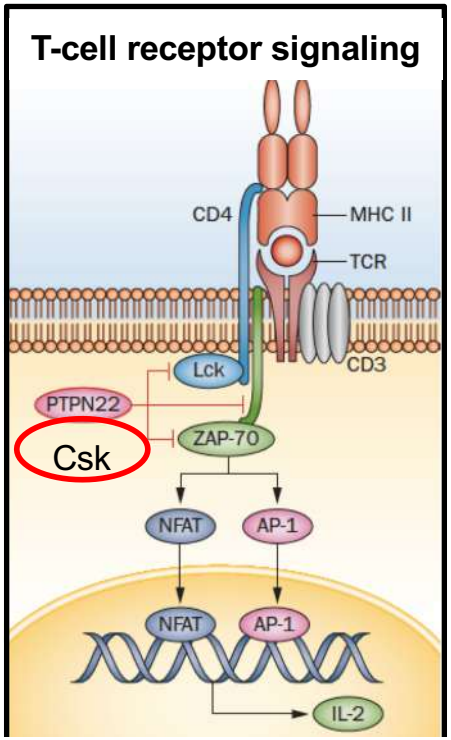


Summary of the putative substrates of PTPN22 in T cells. Established substrates include Y420 and Y394 in activation loops of Src homology (SH) family protein tyrosine kinases (PTKs) **Fyn** and **Lck**, respectively, and the autoactivatory Y493 of **Zap-70.** Putative PTPN22 substrates that undergo tyrosine phosphorylation upon TCR engagement include (a) TCR-CD3ζ and CD3ε, (b) the guanine nucleotide exchange factor (GEF) VAV, and (c) the ATPase valosin-containing protein (VCP) (11). Y75 of Src kinase–associated protein homolog (SKAP-HOM) is a recently described T cell substrate for PTPN22

Struttura schematica di PTPN22 e sua interazione con Csk e TRAF3







PTPN22 displays three major domains:

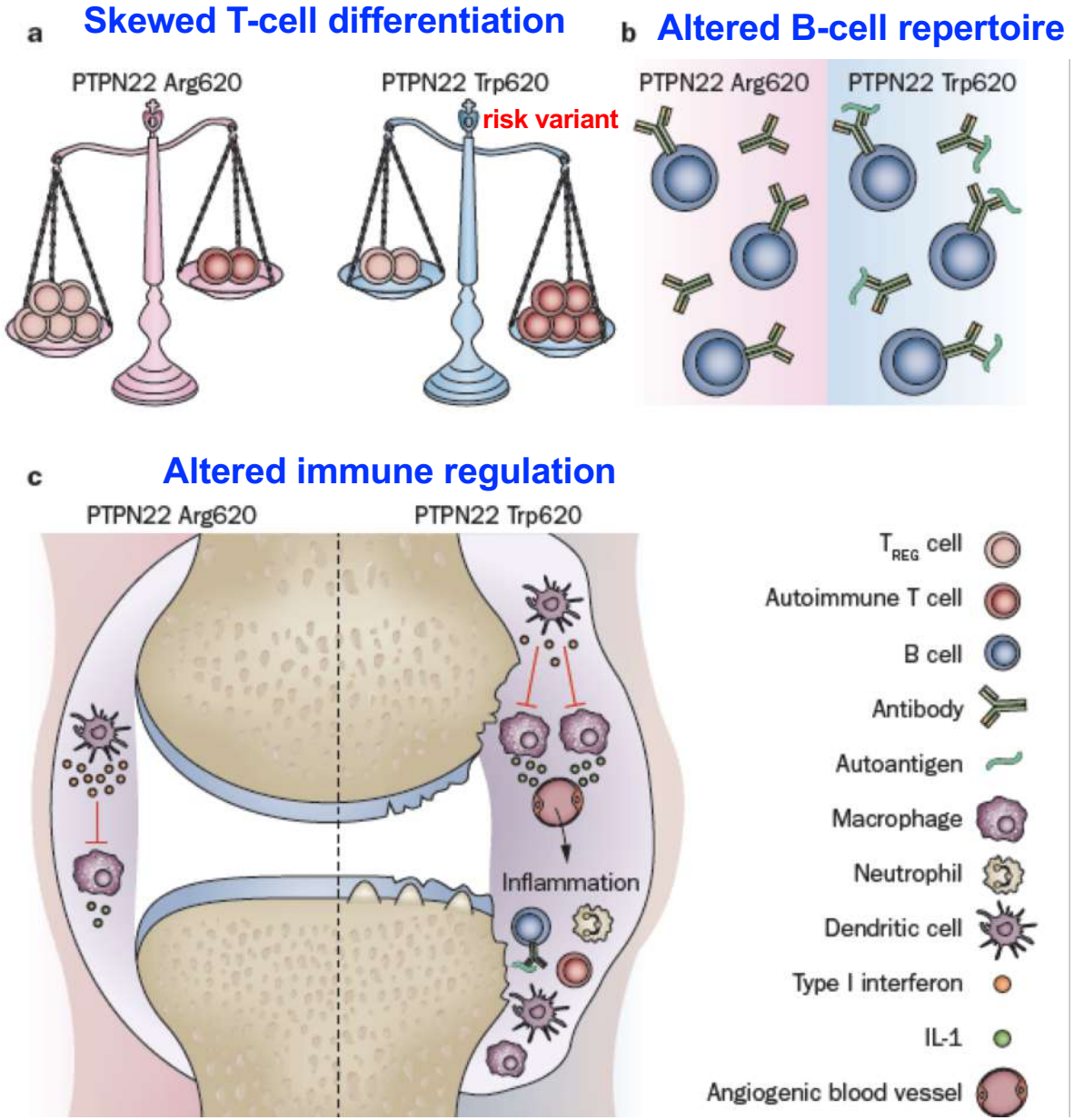
1. N-terminal/PTP domain (amino acids 1–300),
2. interdomain (amino acids 301–600), and
3. C-terminal domain (amino acids 601–807) with high conservation (>90%) between human PTPN22 and murine Ptpn22.

Areas of high conservation are indicated in blue. Residues D195 and C227 are critical for catalytic function. PTPN22 forms a high stoichiometry complex with **Csk** through interaction between the P1 motif in the C-terminal domain of PTPN22 and the SH3 domain in the N terminus of Csk. PTPN22 also interacts directly with TRAF3 in myeloid cells, but it is unclear whether a ternary PTPN22-Csk-TRAF3 complex is formed (*question mark*). Also shown are PTPN22 phosphorylation sites at S35 and Y536 (in *red*). A conserved region of the interdomain that constitutively inhibits the phosphatase activity is localized between amino acids 300 and 320.

The R620W variation in the P1 motif of the C-terminal domain decreases binding affinity between Csk and PTPN22 and between TRAF3 and PTPN22.

(Abbreviations: Csk, C-terminal Src kinase; PTP, protein tyrosine phosphatase; TRAF3, tumor necrosis factor receptor–associated factor 3.

Models of PTPN22-regulated autoimmune disease



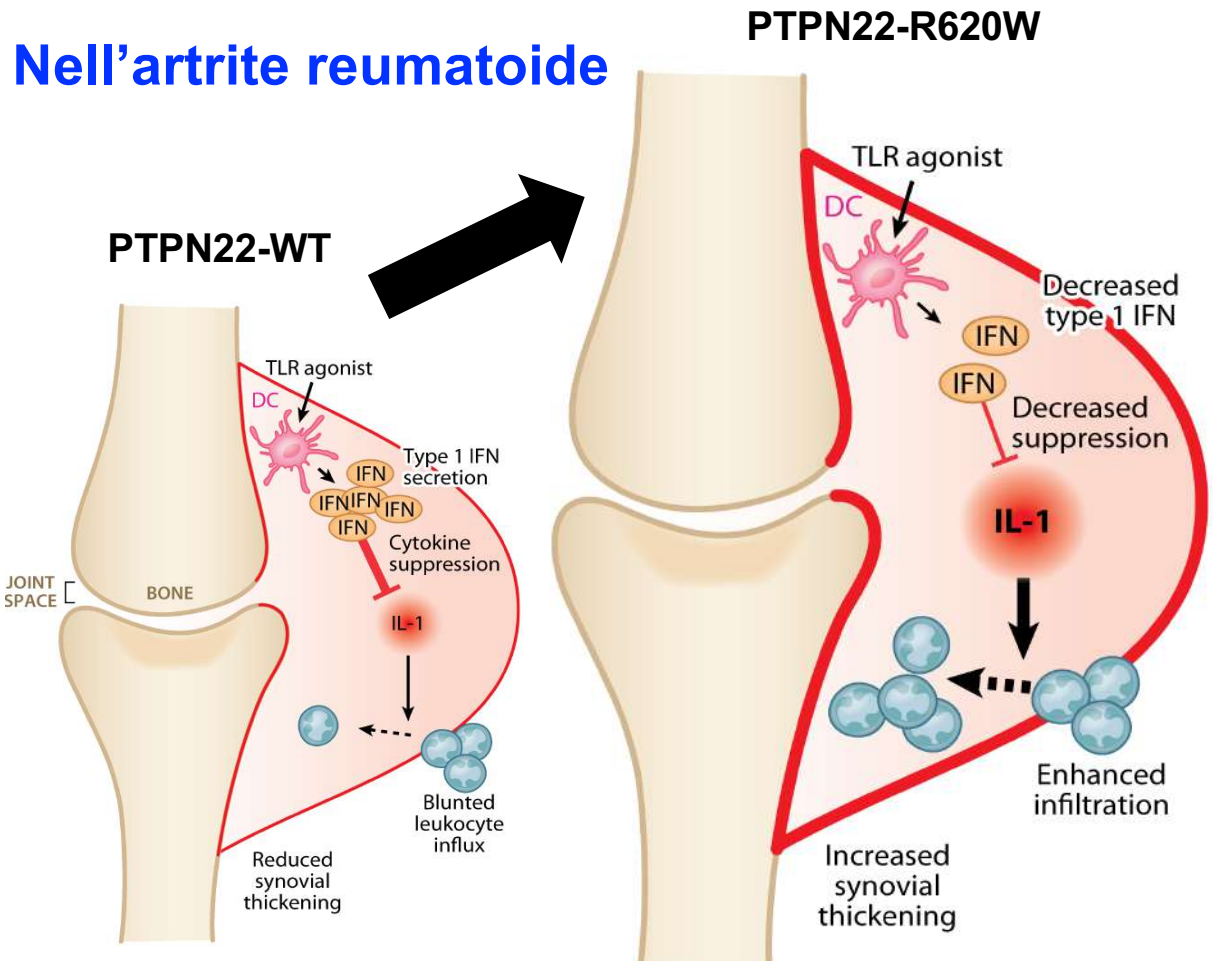
a| The PTPN22 Trp620 promotes the expansion of pathogenic, **autoimmune T cells**.

b| PTPN22 Trp620 alters the **B-cell repertoire,** promoting autoantibody production.

c| PTPN22 Trp620 **impairs type I interferon production by myeloid cells,** which during homeostasis functions to antagonize the effect of proinflammatory cytokines, for example, to protect against arthritis in the synovium.
Abbreviations: PTPN22, Tyrosine-protein phosphatase nonreceptor type 22; TREG cell, regulatory T cell.

Possibile meccanismo patogenetico della variante di rischio PTPN22-R620W

Nell'artrite reumatoide

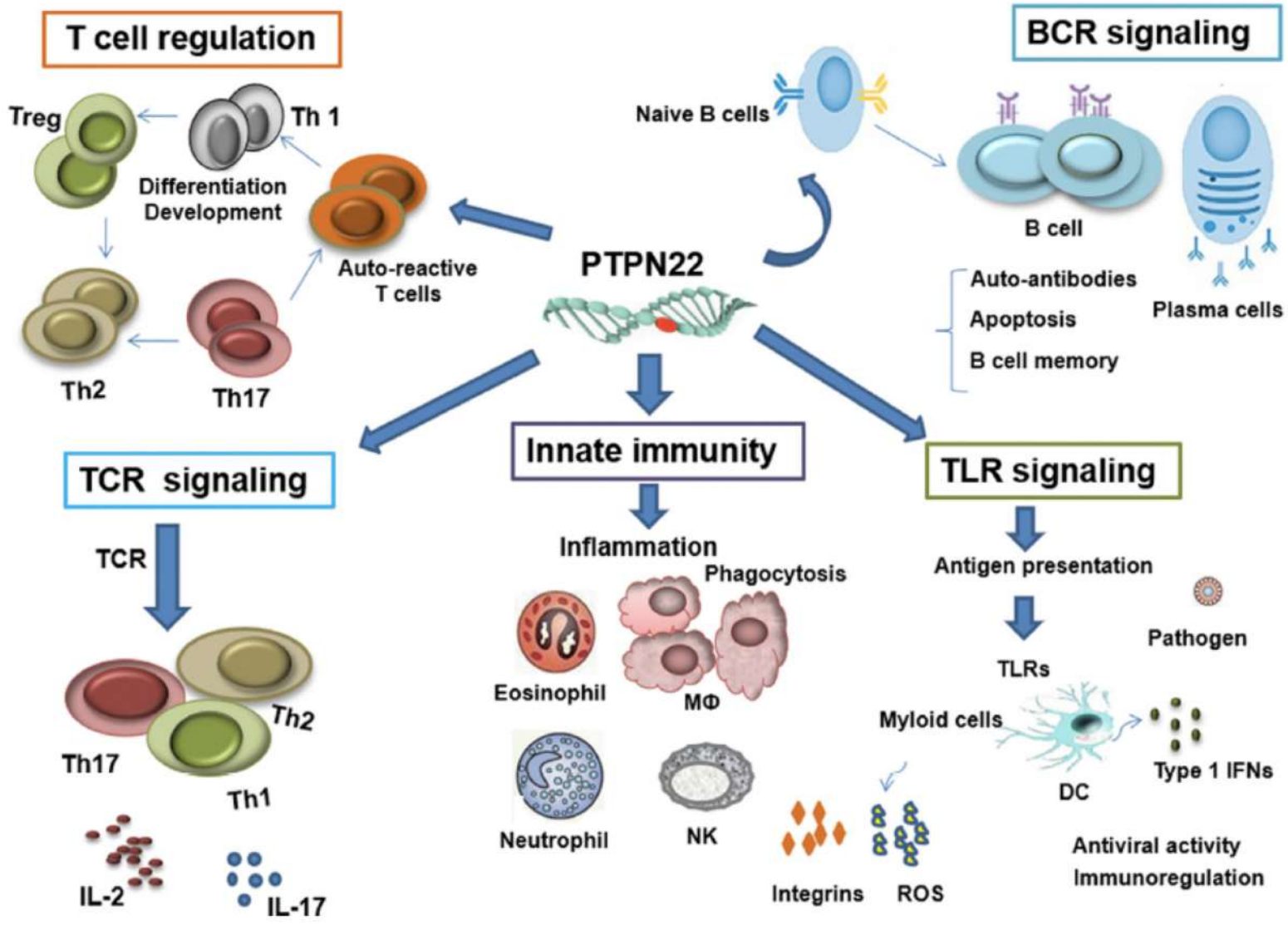


Bottini N, Peterson EJ. 2014.
Annu. Rev. Immunol. 32:83–119

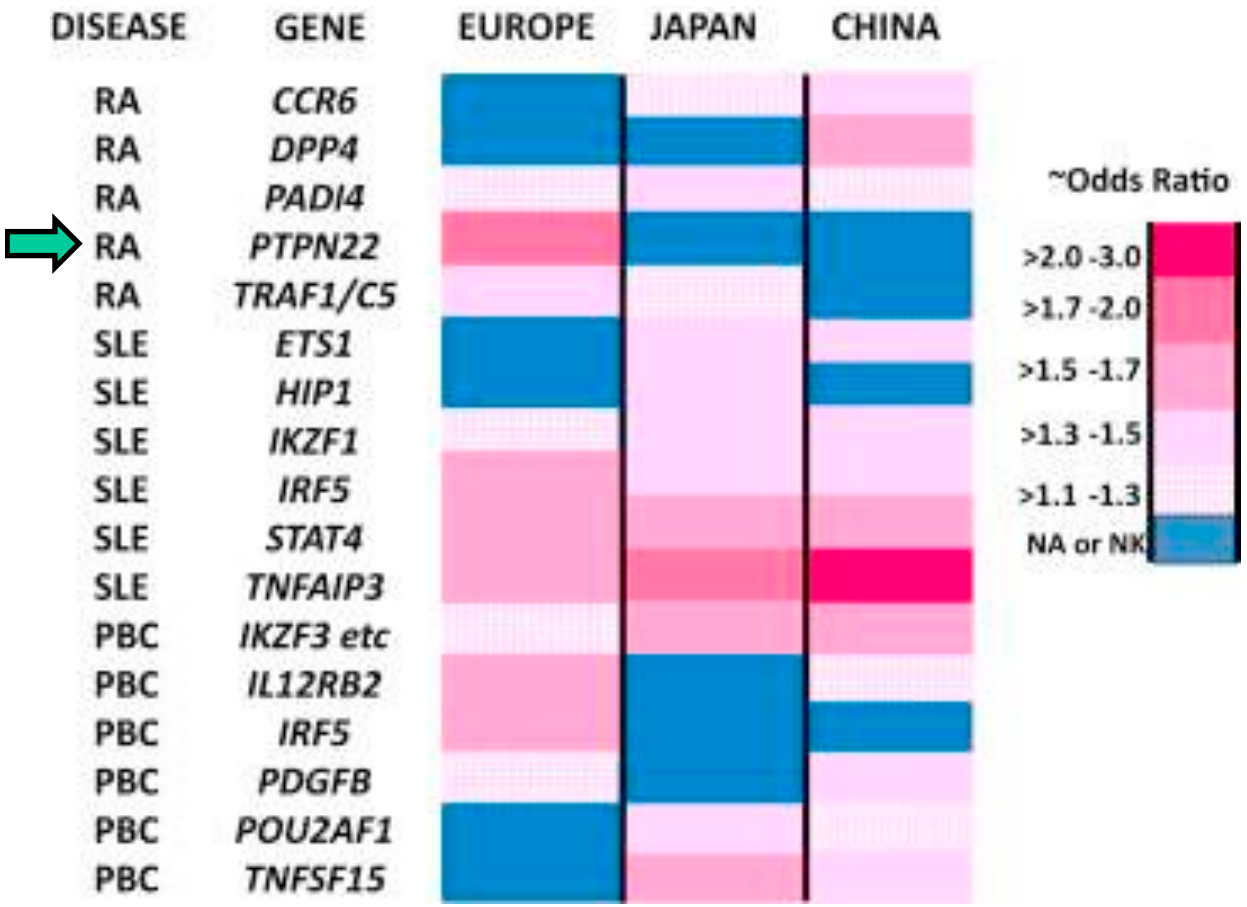
The autoimmunity-associated variant PTPN22-R620W may contribute to disease predisposition through differential regulation of **B cell repertoire** development (not shown), **T cell signaling** (not shown), and **TLR-driven type 1 IFN production.**

TLR signals drive type 1 IFN, which can suppress the activity of proinflammatory cytokines such as IL-1 in inflammatory arthritis. After treatment with TLR agonist, arthritic PTPN22-R620W-bearing animals display reduced synovial type 1 IFN but increased IL-1, leukocyte infiltration, and joint swelling compared with PTPN22-WT-bearing animals. (Abbreviations: DC, dendritic cell; IFN, interferon; TLR, Toll-like receptor.)

Multiple roles of PTPN22 in immune signaling pathways



Comparison of effect sizes for gene polymorphisms in three different population groups in three different autoimmune diseases

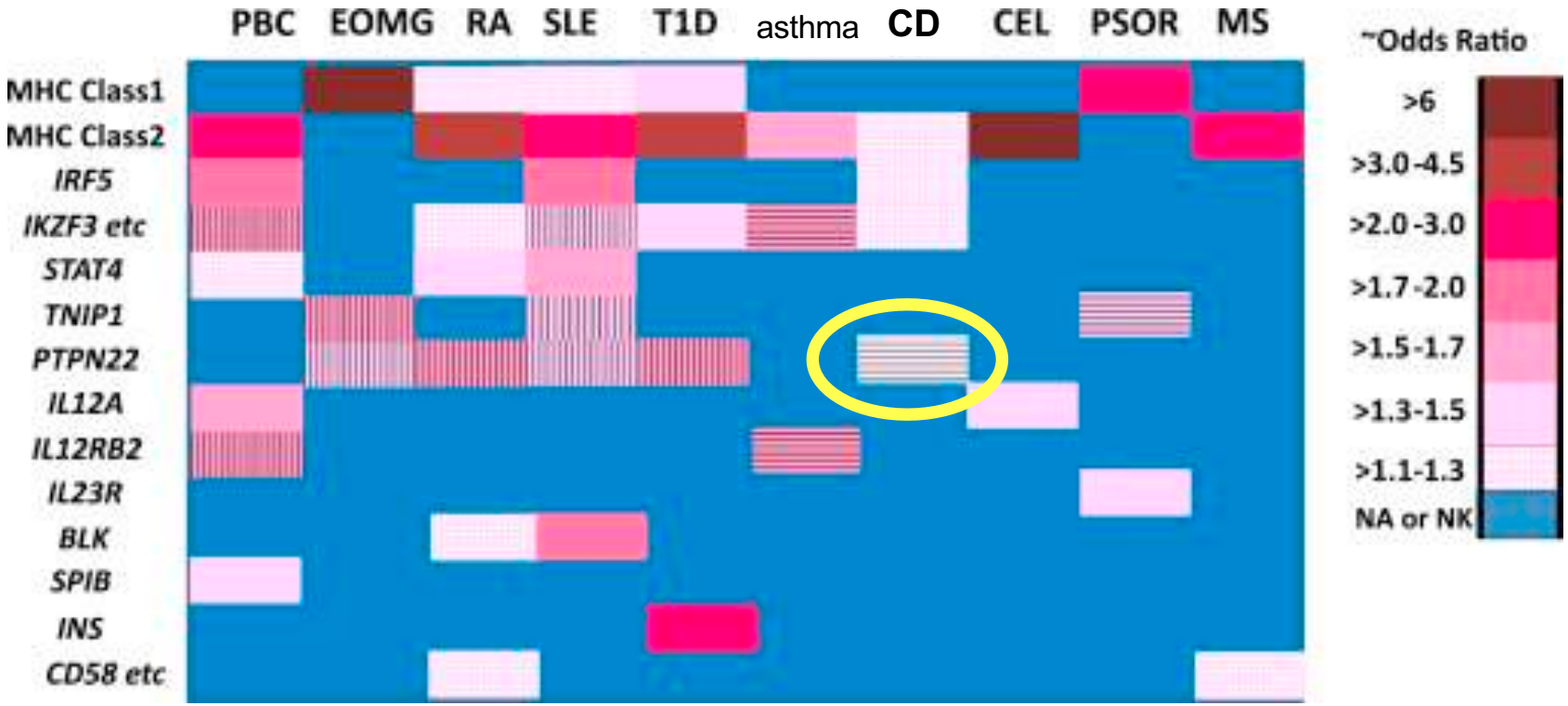


The approximate odds ratios (ORs) are indicated by the color code legend and for each the OR is indicated in the positive direction (regardless of minor allele frequency). The ORs were preferentially chosen from larger studies or meta-analyses. The genes selected were chosen on the bases of showing an OR of >1.3 in at least one of the populations chosen. For some of the genes for which the legend key indicates no association or not known (NA or NK) there may be limited data that suggests possible association that does not meet the standard genome-wide criteria for statistical significance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Ancestry makes a difference !!!

Seldin MFJournal of Autoimmunity, Volume 64, 2015, 1–12

Comparison of effect size for gene associated SNPs in multiple different autoimmune diseases



Comparison of effect size for gene associated SNPs in multiple different autoimmune diseases. Disease abbreviations that are not clear from the main text include: asthma (ASTH), Crohn's disease (CD), celiac disease (CEL) and psoriasis (PSOR). Asthma is included for comparison of autoimmune conditions with another immunologically mediated disease. The approximate odds ratios (ORs) are indicated by the color code legend and for each the OR is indicated in the positive direction (regardless of minor allele frequency). Horizontal or vertical lines are shown over the color coded ORs when the opposite SNP/haplotype is associated with different diseases (e.g. for the IKZF3 etc. in which the asthma association is clearly the opposite of those for PBC (primary biliary cirrhosis) and SLE. The ORs are derived from studies of European ancestry subject sets and are preferentially chosen from larger studies or meta-analyses. For some of the genes for which the legend key indicates no association or not known (NA or NK) there may be limited data that suggests possible association that does not meet the standard criterion ($p < 5 \times 10^{-8}$). Where different SNPs show disparate ORs the association the highest OR is shown. The gene list was selected based on inclusion of most genes with at least moderate ORs (OR > 1.1) in at least one of the autoimmune diseases and is in part biased to emphasize the differences in effect sizes. IKZF3 etc represents a gene cluster that includes IKZF3, ORMDL3, ZPBP2, and GSDMB. CD58 etc. represents a gene cluster including CD58, CD2, and IGSF2.

PBC= cirrosi biliare primaria; EOMG= miastenia grave; CEL= celiachia

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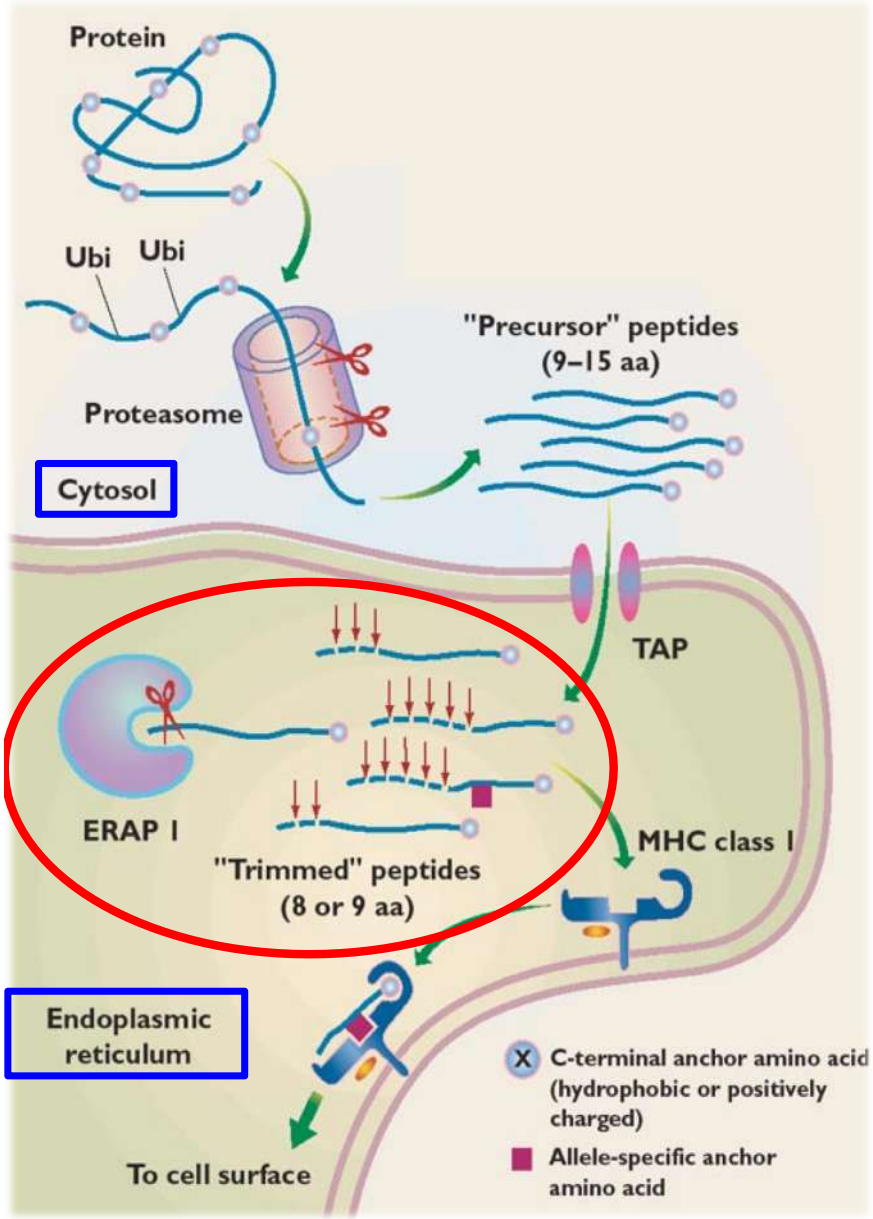
ERAP1 (endoplasmic reticulum aminopeptidase 1) è un fattore di rischio per patologie autoimmuni associate a geni HLA di classe I



Malattia	gene HLA di classe I associato
Spondilite Anchilosante (SA)	HLA-B*27
Psoriasi (PsO)	HLA-C*0602
Sindrome di Behçet (SB)	HLA-B*51

L'associazione di ERAP1 alla SA, PsO e SB si riscontra esclusivamente nei soggetti rispettivamente HLA-B*27, HLA-C*0602 o HLA-B*51 positivi, indicando **un'interazione epistatica** tra geni o

"GENE-GENE interaction"

Dati provenienti da GWAS

Geni associati a malattie autoimmuni

I dati derivano da GWAS (genome wide association studies)

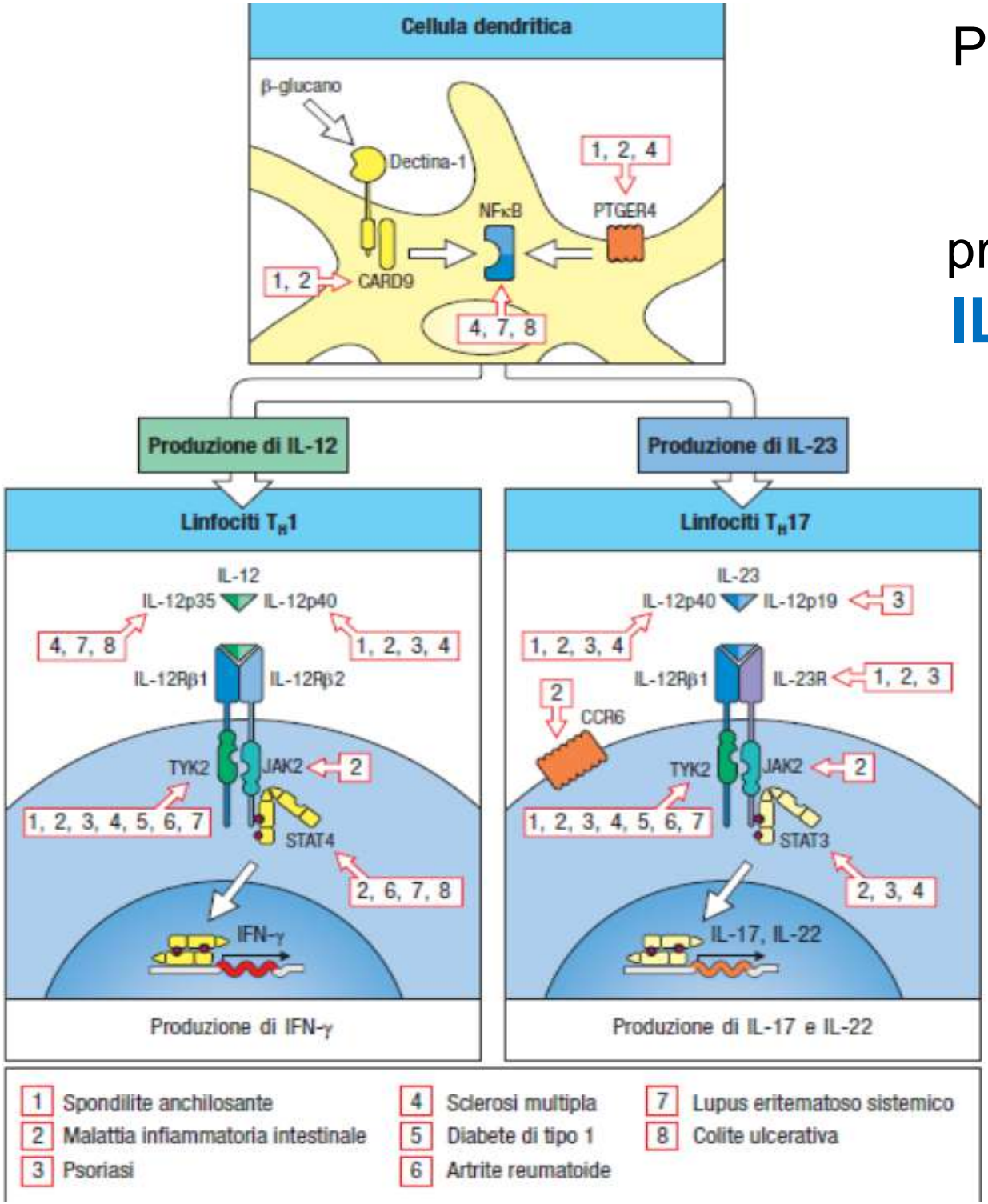
Table 1

Top associated genes in seven common autoimmune diseases

Disease	Gene	Reference
CeD	IL21	[40,41]
	RGS1	[40]
	HLA-DQA1	[41]
MS	HLA-DRB1	[16•,42–44]
	METTL1, CYP27B1	[42]
	CD58	[16•,42]
	HLA-B	[16•]
	TNFRSF1A	[16•]
	IL2RA	[16•,44]
Psoriasis	HLA-C	[45–47]
	IL12B	[45,48]
	TNIP1	[45]
	IL13	[45]
	TNFAIP3	[45]
	LCE3D, LCE3A	[48]
Crohn's	IL23R	[35,38,49–51]
	ATG16L1	[35,38,50]
	PTGER4	[38]
	NOD2	[35,38,49]
	ZNF365	[38]
	PTPN2	[35,38,52]
	NKX2-3	[38,52]
	IRGM	[38,52]
	IL12B	[38]
	MST1	[38,52]
	CCR6	[38]
	STAT3	[38]
	LRRK2, MUC19	[38]
	TNFSF15	[38]
	CDKAL1	[38]
	BSN, MST1	[35]
	CARD15	[50]
RA	PTPN22	[32–35]
	REL	[32]
	OLIG3, TNFIP3	[33,53]
	HLA-DRB1	[33–35]
	HLA-DQA1, HLA-DQA2	[35,54]
	TRAF1-C5	[34]

Table 1 (*Continued*)

Disease	Gene	Reference
SLE	TNFAIP3	[55]
	STAT4	[37,55]
	HLA-DQA1	
	IRF5, TNPO3	[37]
	ITGAM, ITGAX	[37]
	C8orf13, BLK	[37]
	BANK1	[56]
T1D	MHC	[35,38,57,58]
	PTPN22	[35,38,57–59]
	INS	[38,58,59]
	C10orf59	[38]
	SH2B3	[35,38]
	ERBB3	[35,38,57,59,60]
	CLEC16A	[38,57]
	CTLA4	[38,57]
	PTPN2	[38,57,59]
	IL2RA	[38]
	IL27	[38]
	C6orf173	[38]
	IL2	[38]
	ORMDL3	[38]
	GLIS3	[38]
	CD69	[38]
	UBASH3A	[38,61]
	IFIH1	[38,59]
	BACH2	[38,57]
	CTSH	[38,57]
	PRKCQ	[38,57]
	C1QTNF6	[38]
	C12orf30	[57]
	C1QTNF6	[57]
	KIAA0350	[35,58,59]
	C12orf30	[59]



Patologie autoimmuni associate a geni coinvolti nella produzione di **IL-12** e **IL-23** e nei pathways di trasduzione del segnale dei relativi recettori

Genes associated with autoimmune disorders

Chromosome Region	Genes of Interest[a]	Function	Diseases[b]
1p13	*PTPN22*	T and B cell receptor signaling	RA, T1D, CD
2q33	*CTLA4*	Transmits inhibitory signals to T cells	T1D, RA
6p21	*MHC*	Major histocompatibility complex	Most autoimmune disorders
1p13	*CD2/CD58*	Activation of T lymphocytes	RA, MS
1p31	*IL23R*	Unique component of the heterodimeric IL-23 receptor	IBD, PS, AS
1q32	*IL10*	Downregulates immune responses, including cytokines, MHC class II and costimulatory molecules	IBD, SLE, T1D
4q26	*IL2/IL21*	T cell trophic growth factors	CeD, IBD, RA, T1D
5q33	*IL12B*	p40 subunit common to IL-12 and IL-23	IBD, PS
10p15	*IL2RA*	IL-2 receptor α chain	MS, T1D
6q23	*TNFAIP3*	Induced by TNF and pattern recognition receptor activation; inhibits NF-κB signaling	RA, SLE, PS
5q33	*TNIP1*	Interacts with TNFAIP3	SLE, PS
6q21	*PRDM1*	Transcriptional repressor of IFN-β; induces B cell maturation	RA, SLE
8p23	*BLK*	B lymphoid tyrosine kinase	SLE, RA
18p11	*PTPN2*	T cell protein tyrosine phosphatase	IBD, T1D

Genome-wide significant association defined as p value $< 5 \times 10^{-8}$.

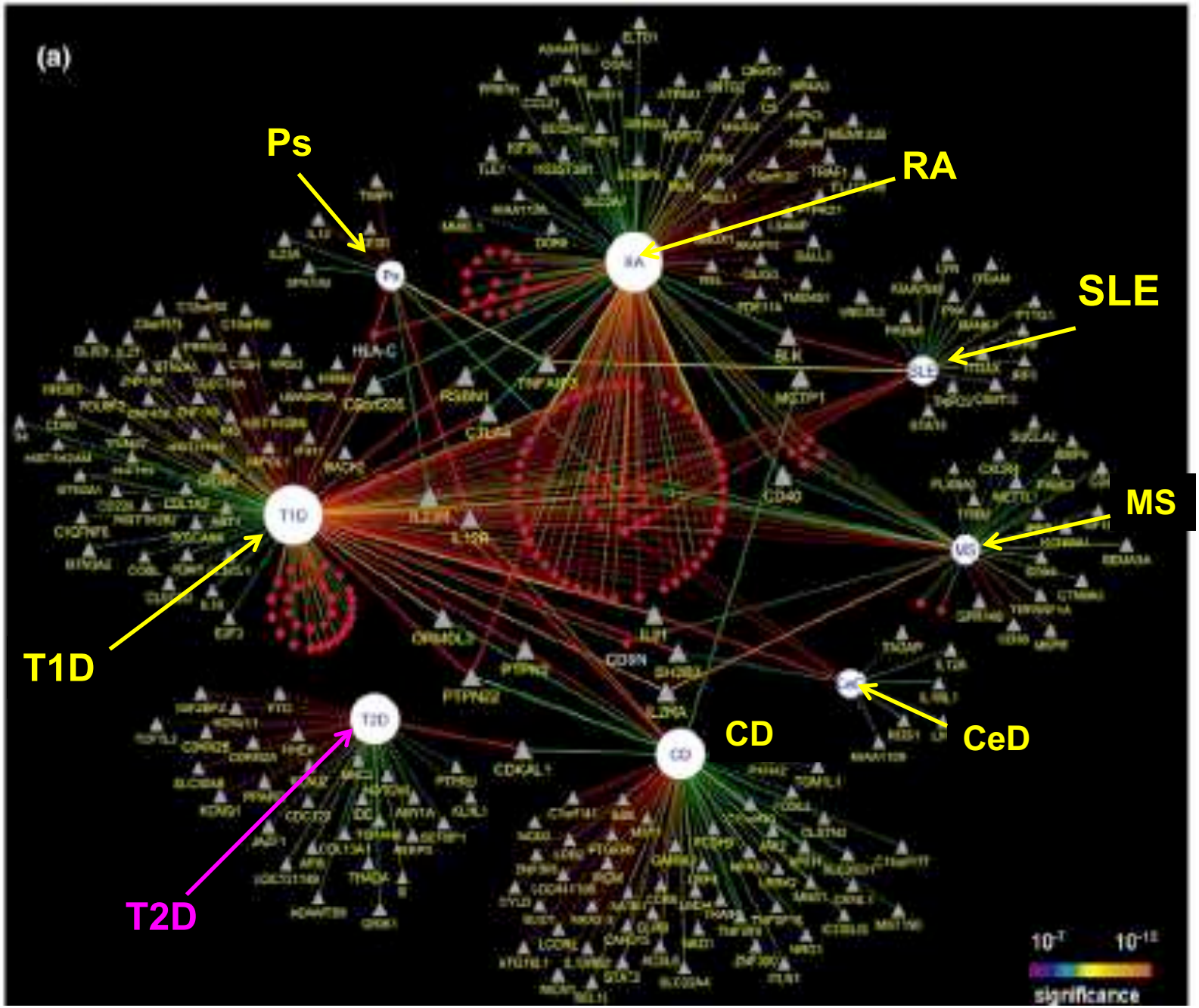
[a]Association regions often encompass either no genes or multiple genes, with the precise causal gene often not definitively established.

[b]AS, ankylosing spondylitis; CeD, celiac disease; IBD, inflammatory bowel disease; MS, multiple sclerosis; PS, psoriasis; RA, rheumatoid arthritis; T1D, type 1 diabetes mellitus.

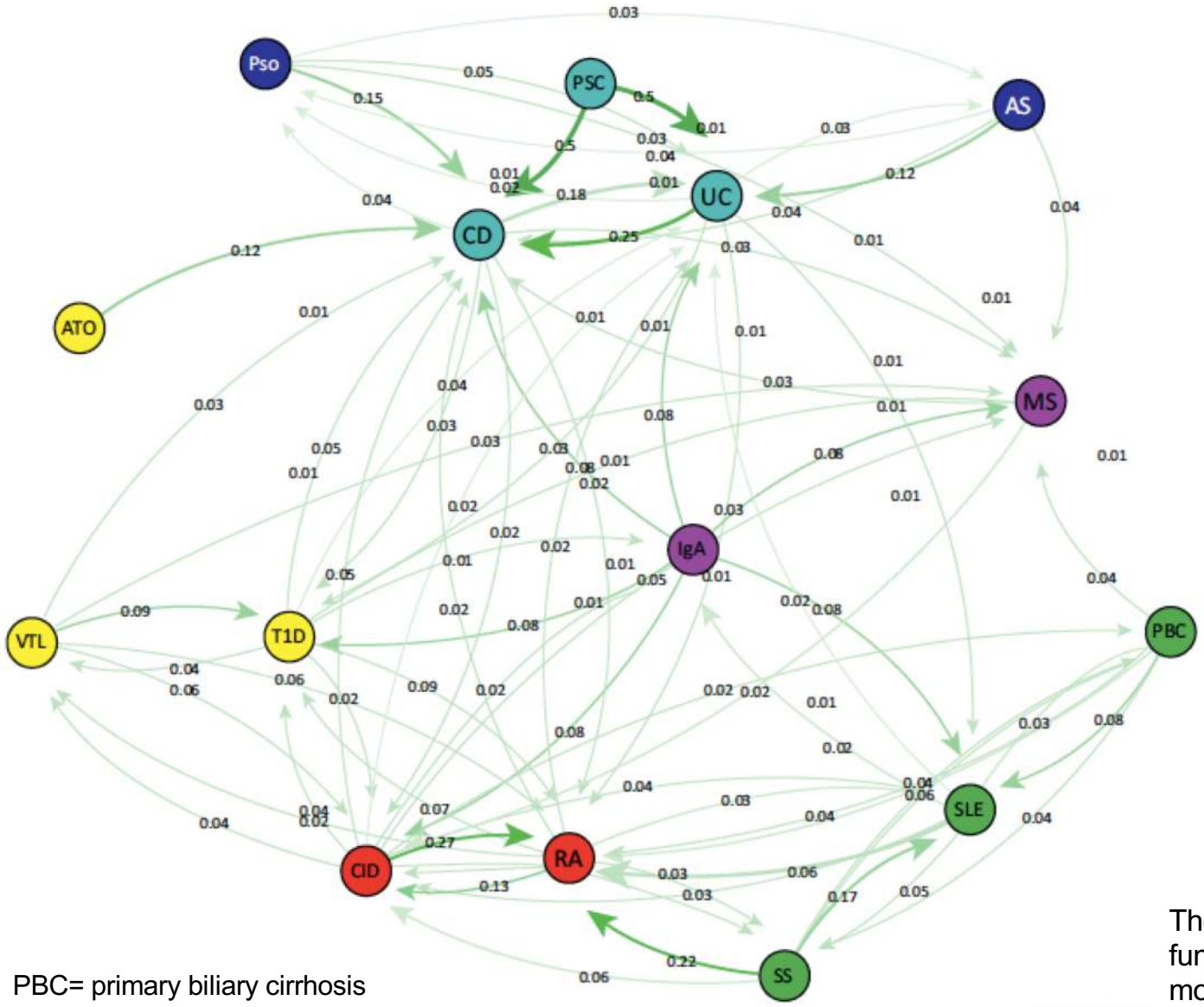
Network tra malattie autoimmuni e geni associati

P value $<10^{-7}$

In rosso i geni della regione MHC



Patterns of risk association across immune-mediated diseases



PBC= primary biliary cirrhosis
PSC= primary sclerosing colangitis
SS= systemic sclerosis
ATO=atopic dermatitis

Data from GWAS catalog (http://www.genome.gov/gwastudies)

Overlapping between pathways and mechanisms involved in immune-mediated diseases

Concept of molecular nosology

TRENDS in Immunology

The relations between diseases as a function of shared associations, using the most recent (september 2012) GWAS catalog. For each pair of diseases, we compute the proportion of associations known for disease 1 that are shared with disease 2 and viceversa (these proportions are not symmetrical).

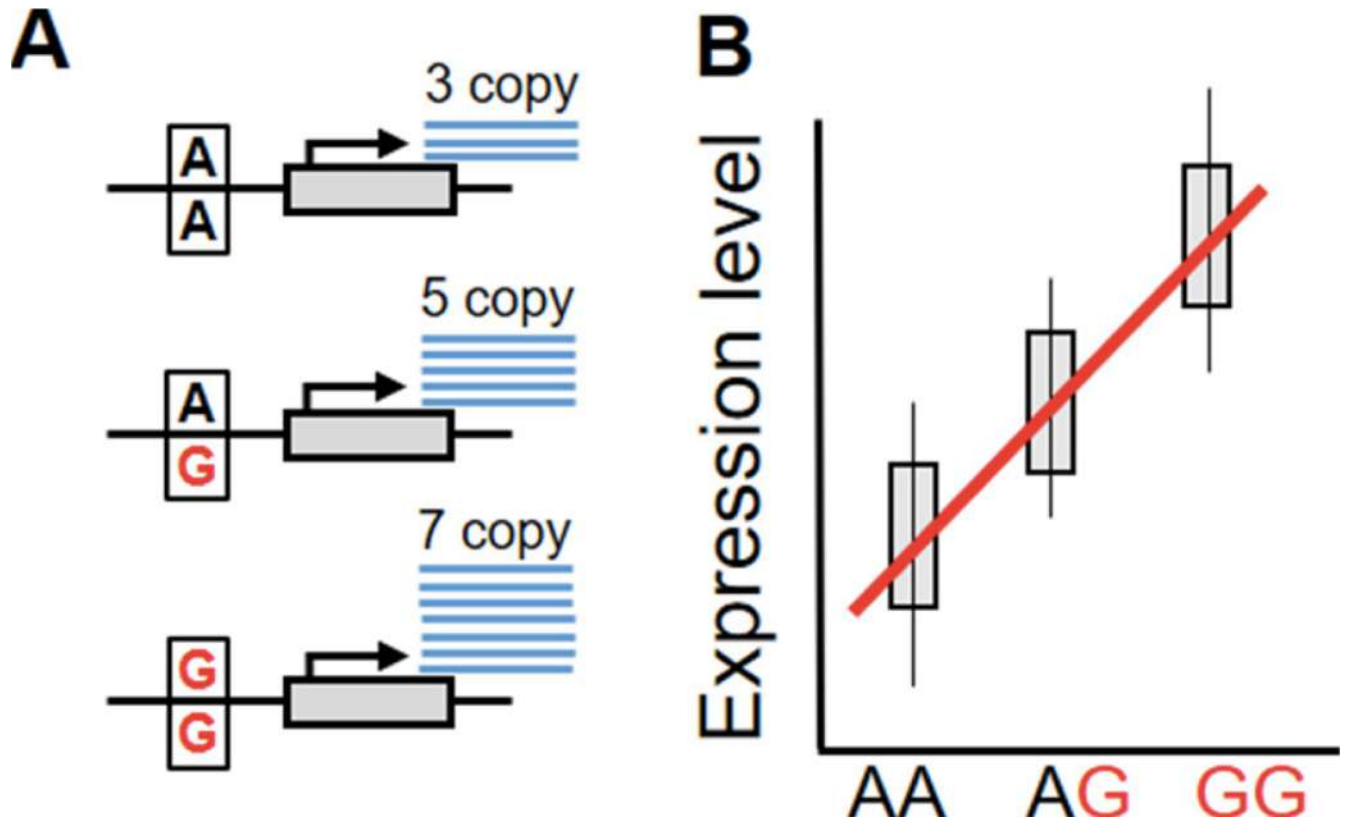
Integration of GWAS to other experimental approaches

Approach	Established and Potential Advances
Genome-wide association studies	Have identified a large number of definitive associations across autoimmunity, with many shared across autoimmune diseases
Search for uncommon DNA variants	May identify more penetrant alleles with larger functional effects
Transcriptome sequencing	Will identify tissue-specific alternative isoforms, noncoding RNAs
Expression quantitative trait loci mapping (eQTL)	Mapping DNA polymorphisms to variable RNA expression
Epigenetic analysis: chromatin modifications	More comprehensive maps of DNA sequences modulating transcriptional regulation
Sequence analysis of the intestinal microbiome	Potentially tractable environment covariate modulating intestinal and systemic immune responses
Humanized mice	Incorporates key human immune response components in model systems
Human immune analyses	Prioritize new therapies, identify disease subtypes, and follow disease course

Interdisciplinary studies as post-GWAS approaches

Genetics-genomics-immunology-infection-bioinformatics

Expression quantitative trait loci (eQTL) analysis



eQTL analysis is a study assessing the association of genetic variants with gene expression levels.
(A) Schematic illustration of eQTL variant. In this example, a eQTL variant has A and G allele, and the G allele increases the expression of a near-by gene (grey box). (B) Schematic illustration of eQTL analysis. Firstly, we sorted samples according to the genotype of eQTL variant (AA, AG, or AG). As illustrated in a boxplot, we usually observe substantial amount of variation in the expression level in each genotype group because non-genetic factors also affect gene expression level. Therefore, relatively large sample size is required when the eQTL effect size is modest. We regress the expression level on the genotype, and estimate the size and the significance of eQTL effect.

Genetic studies of complex autoimmune diseases

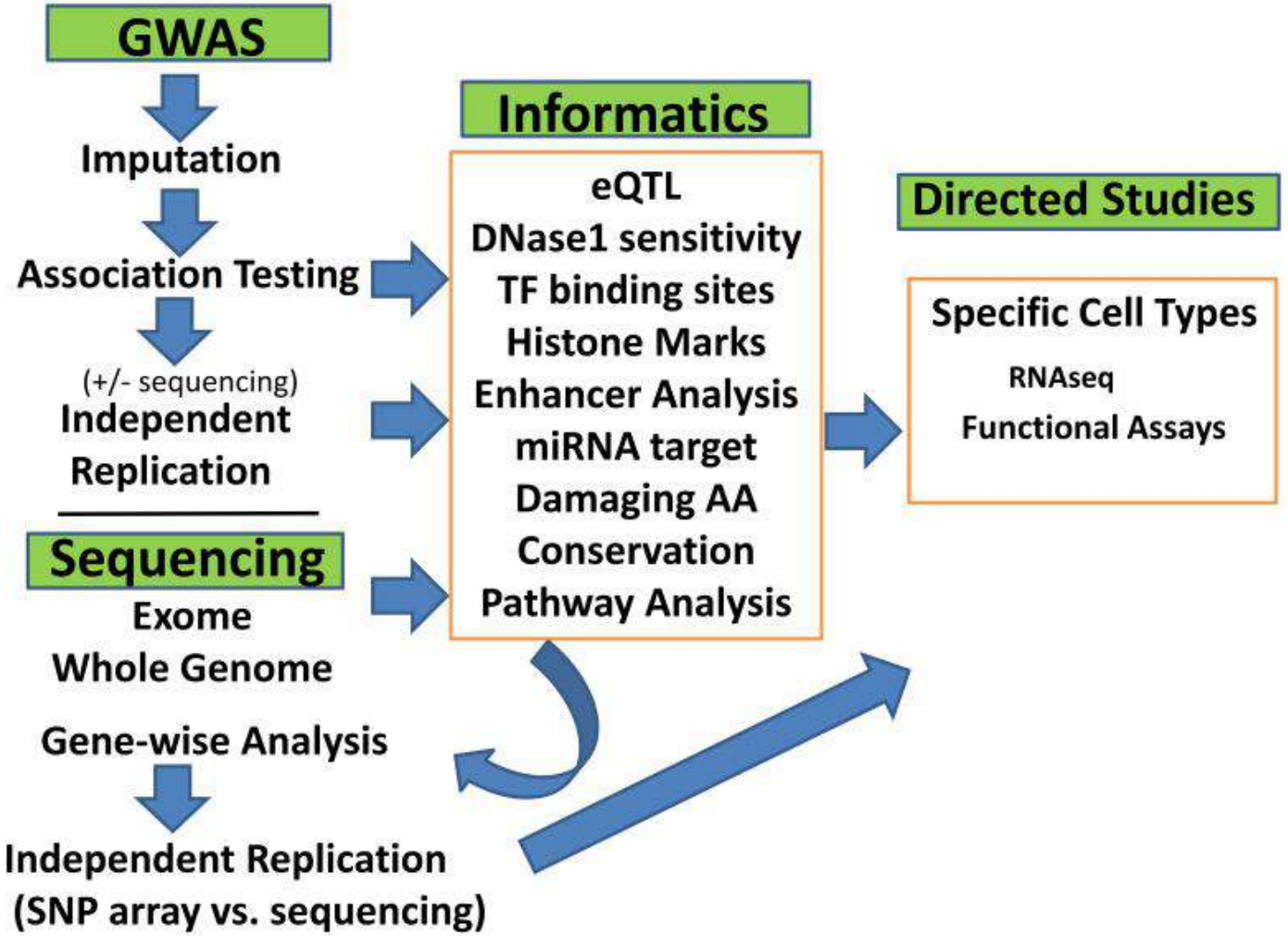


Diagram of general scheme for genetic studies of complex autoimmune diseases. GWAS studies can greatly benefit from imputation and **replication** studies for loci discovered in the discovery phase. For some studies replication studies are limited to those loci (genes) that are also part of **pathways** for genes identified as significantly associated with the disease in previous studies. This is also proposed for sequencing studies to identify less common variants in which power issues may be partially addressed by limiting replication analyses based on prior pathway information.

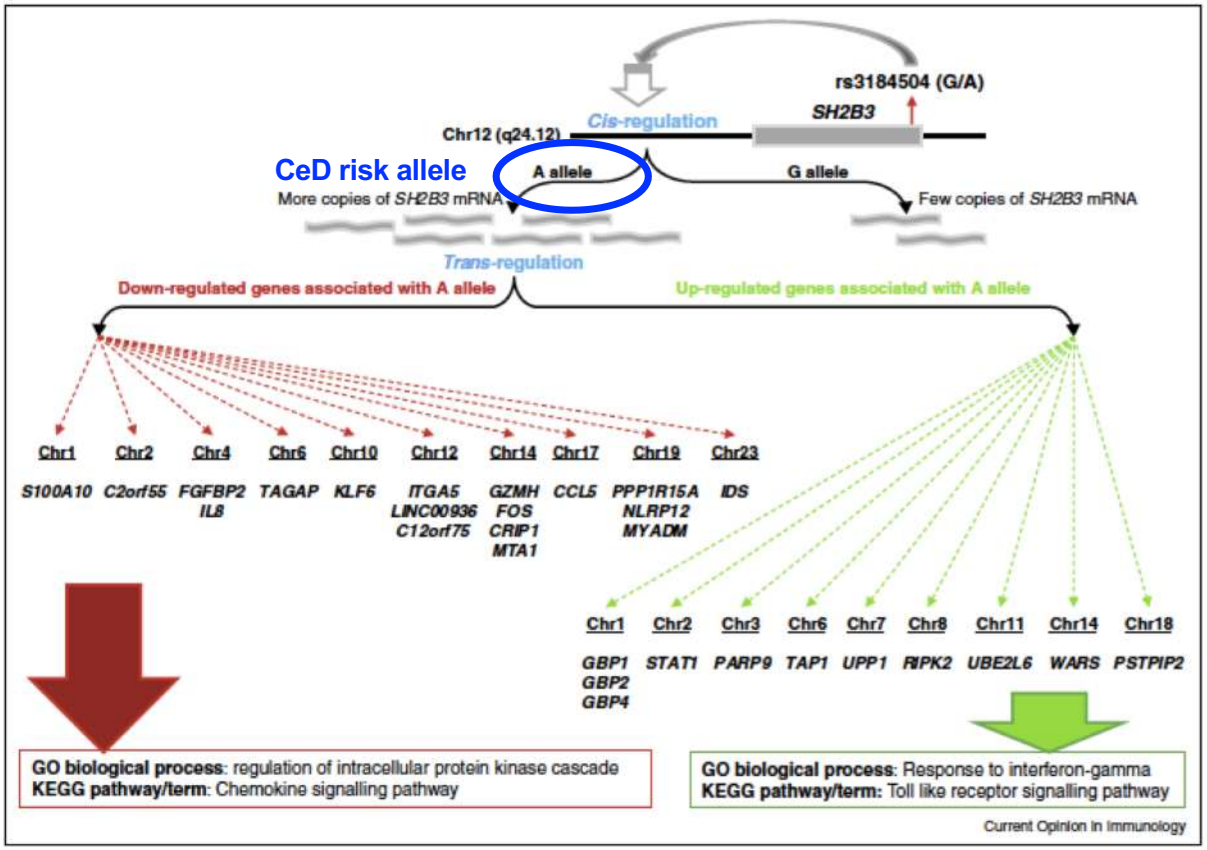
Le patologie infiammatorie croniche immuno-mediate possono essere considerate una conseguenza dell'adattamento del sistema immunitario all'ambiente?

La pressione selettiva esercitata dai patogeni quale effetto ha generato?

Esiste un notevole "overlapping" tra geni implicati nella suscettibilità alle patologie infettive e geni implicati nelle patologie autoimmuni e autoinfiammatorie

Nel corso dei secoli, la selezione naturale ha contribuito alla suscettibilità odierna alle patologie infiammatorie croniche immuno-mediate

Esempio di adattamento del sistema immunitario



SNP rs3184504 on human chromosome 12 is associated with autoimmune diseases. eQTL mapping showed that the autoimmune risk allele (A allele) up-regulates *SH2B3* gene expression (*cis*-eQTL) and also affects 29 other genes on different chromosomes (*trans*-eQTL). Pathway analysis showed enrichment of genes for innate immunity among the up-regulated genes (green dotted arrows), and enrichment of genes for chemokine signalling pathway among the down-regulated genes (red dotted arrows).

Il gene **SH2B3** è associato alla **celiachia** e ad altre patologie autoimmuni.

Questo gene codifica per un adattatore molecolare che regola infiammazione, ematopoiesi,crescita e migrazione cellulare ed è implicato in pathway di signaling di citochine e chemochine. La variante di rischio (R262W) è molto comune tra gli europei ma rara tra gli asiatici e gli africani.

Questa variante è stata selezionata circa 1200-1700 anni fa per il suo effetto protettivo che ha avuto nel corso della pandemia di peste (*Yersinia pestis)*.